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EDITOR'S VIEWPOINT

The Syrian hamster has been used as an experimental animal since about 1930, but its anatomy, physiology, genetics, and its diseases have been investigated only superficially. Wantland (p. 631) has added a valuable chapter to the knowledge of the hamster by his intensive study of the parasitic fauna of animals from 8 different sources. In addition to discussing the effects of the parasites on the animals, the possible danger of infection of laboratory workers by many of these parasites is emphasized. While Wantland directs attention to the hazard of licking labels in the laboratory, the more dangerous habit of eating and drinking beverages in or near the animal quarters is not discussed. Too many workers in the biologic sciences have adopted the habit of afternoon tea from their safer colleagues in the physical sciences. The animal laboratory is a place which presents hazards that must not be treated lightly.

Hamsters and ferrets, among other animals, develop salivary calculus. King, Rowles, Little, and Thewlis (p. 650), examining calculus from the teeth of these animals, clinically, and by x-ray diffraction, found the animal material less highly calcified than that from human subjects and found no material other than hydroxyapatite in the animal calculus. The need for correlated histologic and histochemical studies is pointed out. The presence of salivary calculus and dental caries in hamsters suggests the opportunity for further study of their interrelationship.

From a further study of estrogen and androgen effect on rat caries, Muhler and Shafer (p. 661) conclude that androgens have no significant effect on rat dental caries activity and that estrogens increase the incidence of their carious lesions. This latter effect is more pronounced in gonadectomized animals. It is interesting to observe the variation in the numbers of carious lesions (6.2 to 9.2) in their 6 control groups of 13 to 18 animals each. The experimental groups of similar size show from 7.8 to 10.2 lesions. One must be extremely careful in interpreting variations of these magnitudes in small groups of animals.

The publication of the Proceedings of the International Association for Dental Research (33rd General Meeting) presents an opportunity to review the abstracts of 173 papers read at the meeting, 37 papers presented to the Dental Materials Group, and an additional 54 abstracts read by title. The great numbers of papers read at the annual meetings of the International Association for Dental Research present a problem to the interested investigator and scholar who wish to follow the advances being made in other laboratories. The meetings have been sectionalized for several years and no individual can hear all, or even most, of the papers. This year, the abstracts were prepublished partly for the purpose of allowing selection of papers which the individual attending the meeting most highly desired to hear in detail.

It appears to us that it may be necessary to change the method of selection of papers for presentation at the International Association for Dental Research. Of course, the selection of papers remains the function of the Program Committee, but it might be suggested that each laboratory be limited to one or two papers. At the present time each author is limited to one paper, but in some laboratories many individuals present papers on various phases of the same subject or, in other instances, technicians present papers evading, in spirit, the one-paper-per-author rule. In most instances one would not expect more than one or two significant or reportable papers to come from the average research laboratory each year. However, this is merely a matter of opinion and another viewpoint is that an opportunity is presented to hear reports on work in progress and to hear papers that may never be presented except in brief form as presentations at the meeting.

It is most encouraging to note that 261 members and 351 guests attended this annual meeting. To those who founded the Association this great interest probably represents something beyond their greatest hopes and dreams.

We, again, have the opportunity of publishing the Proceedings of the British Division of the International Association for Dental Research. The British Division held a two-day meeting at the London Hospital Dental School and the 25 papers presented at that meeting are recorded on page 666. It is interesting to note that the British Division was attended by 32 members and 32 guests. We hope that the British Division will continue its growth and its influence on the expanding dental research program in Great Britain.

H. B. G. R.

PARASITIC FAUNA OF THE GOLDEN HAMSTER

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ACCORDING to Adler (1948), the first golden hamsters, *Cricetus auratus* (Waterhouse, 1839), used as experimental animals were descendants of 8 hamsters captured near Aleppo in Syria in 1930. These animals were used in studies on kala-azar at Hebrew University in Jerusalem. Progeny of these hamsters were later sent to England and France for use in various experimental investigations. The golden hamster was introduced into the United States in 1938, and has rapidly become a preferred animal in many types of research. Various persons have gone into the business of raising hamsters, and the research worker or the hamster fancier today can readily procure, from dealers and laboratories, ordinary brown hamsters, a spotted variety called panda hamsters, or true albino hamsters. These latter two varieties have been produced in this laboratory, the albinism behaving apparently as a homozygous recessive.

The increased popularity of the golden hamster in recent years as an experimental laboratory animal has naturally led to an increased interest in and need for intensive studies on the anatomy and physiology of this animal, its susceptibility and resistance to infections and other disorders, care, housing, breeding, natural and accidental parasites, and the development of special techniques in the various kinds of experimental procedures in which the hamster is employed. It is, indeed, most important to learn as much as possible about the total nature of any species of experimental animal because of the possibility of unknown or poorly understood factors markedly influencing experimental results in any given study.

The golden hamster in the experimental laboratory, like many other rodents, harbors a variety of parasitic protozoa, and occasionally plays host to helminths and arthropods without regard for the plans of the research investigator. The part played by animal parasites, engaged in their normal life cycles, upon experimental animals used in various studies, has presented a need for intensive investigations for a long time. Too often parasitism goes either unnoticed or is thought to be not of sufficient import to merit the consideration of the investigator. More recently, students in other areas of investigation than parasitology have become more cognizant of and interested in the effects of parasitism on various hosts used in animal experimental studies. This should lead to better controlled experimentation, further study and better understanding of the

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action and control of parasites, improved preventive measures against infection of laboratory workers, and a greater knowledge of the specific effects of parasitism.

The work reported here was undertaken with three objectives in mind: (1) to bring together into one reference source data from some reports by other workers on animal parasites which have been found in the golden hamster, (2) to describe and discuss briefly the various parasitic species found in a recent examination of 412 hamsters from several sources,* and (3) to indicate precautionary measures against possible infection of laboratory workers by certain parasites occurring in hamsters.

PROTOZOA IN THE GOLDEN HAMSTER

Toxoplasma, small crescent-shaped and oval forms, have been reported from the hamster by Frenkel (1951). These forms usually are from 6 μ to 12 μ in length, possess a central nucleus, and occur in pairs or groups of pairs. The method of multiplication is uncertain although indications are that binary fission occurs. This parasite has been observed free in the reticuloendothelial system and circulating blood, in white blood cells, liver, spleen, lung, brain, and subcutaneous tissues of various mammals. Chandler (1952) stated that it is probable that all forms of mammalian Toxoplasma belong to one species, *Toxoplasma gondii*, which has a cosmopolitan distribution. The natural method of transmission is not known although infections can be transmitted by ingestion of flesh or feces containing the form, and by prenatal infection. The majority of cases of recognized toxoplasmosis in human beings have been fatal brain infections in infants, apparently acquired prenatally. The organisms are difficult to find in many instances. No reliable treatment or prophylaxis is known, and, according to Manwell, Coulston, Binckley, and Jones (1945), Toxoplasma is still one of the least understood of protozoan parasites.

Susceptibility of the golden hamster to *Leptospira icterohaemorrhagiae*, the causative organism of human infectious jaundice or Weil's disease, as well as to a closely related form, *Leptospira canicola*, which causes a high mortality in dogs, has been aptly demonstrated by Morton (1942) and Larson (1944). During the first 2 or 3 days following infection, leptospiras may be demonstrated by dark-field examination of the peripheral blood and can be observed in the urine after 10 or 12 days. These organisms are capable of invading the bodies of experimental animals, as well as man, through mucous membranes, eyes, skin abrasions, and contaminated drinking water. They are extremely slender, coiled forms, 5 μ to 20 μ long, with tapered ends which frequently appear to be hooked or looped back over the body. Dogs not uncommonly harbor leptospiras. Extreme care should always be taken by research workers to guard against acquiring infection, and hamsters not intended for experimental studies on leptospirosis should be housed separately from animals known to have, or

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suspected of having, the infection. Penicillin seems to be the only drug which is reasonably successful in the treatment of Leptospira infections, and immune serum from homologous strains is regarded as somewhat beneficial.

The golden hamster was first used in experiments on kala-azar by Adler and Theodor (1931 to 1938) at Hebrew University. Although a peculiar susceptibility is exhibited by the hamster to experimental Leishmania infection, natural infections seldom occur, according to Bruce and Hindle (1934). Fulton (1944), Harrison and Fulton (1946), and Watson (1947) have reported the hamster to be an excellent animal for studies on leishmaniasis. *Leishmania donovani,* upon which most work has been done, is a small, round form from 2 μ to 4 μ in diameter as it occurs in the human being and experimental animals. An eccentric nucleus is present and a kinetoplast can usually be seen in stained preparations as a minute dot close to the nucleus. Elongated forms are frequently found in the spleen. When cultured or when observed in the digestive tracts of insect intermediate hosts (Phlebotomus, sandflies), these parasites transform into active, flagellated forms. Leishman bodies are found widely distributed in animals experimentally infected. They are especially abundant in the large endothelial cells of blood vessels and lymphatics, spleen, liver, and bone marrow, and in cells of the intestinal wall and mucosa of the oral and nasal cavities. They can usually be found both inside and outside of tissue cells, and occasionally in the circulating blood, nasal secretions, urine, and feces. While natural infections are usually transmitted by the bites of sandflies, experimental animals can be infected by mouth and by spraying the nose with infected secretions. Laboratory workers involved in experimentation on leishmaniasis should exercise great care in avoiding contact with urine, feces, and nasal secretions of infected animals. Chandler (1952) stated that in South America and the Mediterranean region dogs are frequently infected, and transmission of leishmaniasis from dog to man probably occurs more often than from one human being to another. Fulton (1944) found stilbamidine in 10 intraperitoneal injections (20 mg. per kilogram of body weight) highly effective in treating hamsters experimentally infected with kala-azar. While some of the newer aromatic diamidines have proved quite effective, they are also much more toxic than pentavalent antimony compounds such as neostibosan, solustibosan, and urea-stibamine in specific treatment of kala-azar.

It is well established that rodents, as well as other animals in nature, commonly harbor species of Mastigophora and Sarcodina. Many of these forms show little host specificity, and a high degree of transfaunations in rodents has been demonstrated by Saxe (1950). Wenrich (1946), who has done a prodigious amount of work on these forms, especially trichomonads, is inclined to consider most of the protozoa found in the intestine of the hamster to be identical with those in rats. However, the evidence thus far is not conclusive and much remains to be done in the way of transfaunation studies and tests of host specificity of these forms. Wenrich (1946) reported, from observations made on one hamster, the finding of a *Trichomonas muris*-like form, as well as flagellates resembling *Trichomonas minuta, Trichomonas wenyoni, Giardia muris,* and

Chilomastix bettencourti. Wenrich and Saxe (1950) reported success in infecting 2 trichomonad-free hamsters with a small trichomonad originally obtained from a vole, *Microtus pennsylvanicus.* The name *Trichomonas microti* was proposed for this flagellate by these authors and was first described by them.

Examination of 412 golden hamsters by the writer during the past 14 months from the sources mentioned earlier revealed the following protozoa, their location, and incidence: trichomonad flagellates in the intestine, particularly the cecum (99.27 + per cent), *Endamoeba muris* in the cecum (33.0 per cent), *Chilomastix bettencourti* in the cecum (9.22 + per cent), and a form resembling *Giardia muris* in the small intestine (12.37 + per cent). Animals in which trichomonads were observed harbored *Trichomonas muris, Trichomonas wenyoni, Trichomonas minuta,* a form resembling *Trichomonas microti,* and a trichomonad apparently unlike any reported in the literature. *Trichomonas muris* was prevalent in all infected animals with smaller numbers of the other species interspersed in the wet smears of material prepared for microscopic examination.

In Table I are indicated some of the characteristics of these species of trichomonads. Detailed morphologic descriptions can be found in textbooks of protozoology, and in the original publications of Wenrich (1946), Wenrich and Nie (1949), and Wenrich and Saxe (1950).

TABLE I

	T. MURIS	T. WENYONI	T. MINUTA	T. MICROTI
Size	16μ-26μ long 10μ-14μ wide	6μ-16μ long 2.5μ-6.0μ wide	4μ-8.5μ long 2μ-4.5μ wide	4μ-10μ long 2μ-4.5μ wide
Shape	Thick pear-shaped	Lemon- or pear-shaped	Small lemon- or pear-shaped	Small lemon- or pear-shaped
No. ant. flagella	3	3	3	4
No. waves in undulating membrane	5-6	2-3	3	3-4
	Body length membrane	Short membrane ½-⅔ body length	Body length membrane	Body length membrane
Post. flagellum	Present	Present	Present	Present
Costa	Heavy; thick; full length of body	Slender; obscure	Narrow; slight; full length of body	Narrow; slight; full length of body
Axostyle	Broad; hyaline; short posterior protrusion from body	Compact; tapering; posterior protrusion from body	Broad; hyaline; short posterior protrusion from body	Relatively broad; long, pointed posterior protrusion from body
Nucleus	Anterior; oval	Anterior; round or oval	Anterior; round or oval	Anterior; round
Parabasal body	Thick rod- or club-shaped	Slight; delicate	Short; slender	Rod- or Y-shaped
Blepharoplast	One present; small	One present; relatively large	One present; relatively large	One present; small
Metachromatic granules	Heavy, course granules along costa and perinuclear	Heavy, course granules anterior area	Sparsely located along costa	Sparsely located perinuclear
Chromatic ring	Not distinct	Absent	Present	Absent

The fifth trichomonad observed in this study was a more elongated, slender form measuring from 12 to 25 μ long by 5 to 10 μ wide. This form possesses 3 anterior flagella, a heavy, full body-length costa, an anteriorly located nucleus,

and a relatively long, club-shaped parabasal body. Metachromatic granules are heavy and numerous. A bulbar swelling of the posterior end near the point where the broad axostyle emerges together with the shape of the body differentiates this trichomonad from the other forms described.

Multiplication of these trichomonads occurs by binary fission, and occasionally a somatella is observed, indicating multiple fission with merozoite formation. Considerable difference of opinion exists among parasitologists (Wenyon, 1926; Hegner and associates, 1938; and Chandler, 1952) with reference to the formation of cysts by trichomonads. In this study cystlike bodies were definitely observed in all forms of trichomonads obtained from hamsters. These spherical bodies, measuring from 7 μ to 13 μ in diameter, were noted periodically in small numbers in normal fecal pellets, and in excessive numbers in feces from hamsters fed a sunflower seed diet for 3 days or given stale bread soaked in a saturated solution of magnesium sulfate. The writer is inclined to look upon these bodies as true cysts because of their appearance, method of formation, and quiescence. In wet preparations from the cecum, the trophozoite forms can be observed to pass into the cystic stage as the preparation dries. The active forms thrash their flagella wildly about, and the wavelike movements of the undulating membrane increase in speed for a time. All movements gradually become slower, and finally a few spasmodic waves of the undulating membrane seem to envelop the posterior part of the axostyle, pulling it into the cyst. In about one hour, the quiescent cysts are fully formed. Trophozoites placed on a slide with artificial gastric juice encyst within a matter of minutes. Then, upon the addition of 2 or 3 drops of a 5 per cent aqueous solution of pancreatin, movements can again be observed. The organism begins to revert to the trophozoite form first, by a twisting, churning action within the cyst wall. As the action of pancreatin proceeds, the whole organism rotates within the cyst and movements of the undulating membrane can be seen. During a period of 1 to 2 hours following the addition of pancreatin, the undulating membrane increases its movements, the body of the organism assumes various shapes, flagella and the posterior part of the axostyle appear, and, finally, the trophozoite exhibits the characteristic movements of this stage of development.

Hamsters probably reinfect themselves by eating their own feces-containing cysts, and continued fission in the small intestine and cecum results in tremendous numbers of trichomonads. Marked hyperemia of the intestinal wall with pinpoint enterorrhagia was observed in all heavily infected animals, and erythrocytes were demonstrated in many trophozoites. A number of the trichomonad flagellates in various hosts have been suspected of being pathogenic from time to time by research investigators. *Trichomonas vaginalis* is certainly the cause of a vaginitis in the human being characterized by an abundant frothy white discharge. The pathogenicity of *Trichomonas hominis* has not been proved although the infection in man is often associated with persistent diarrhea (Chandler, 1952). Kofoid (1929) and Beatman (1933) reported finding *Trichomonas tenax* associated with advanced inflammatory pyorrhea in human beings. There remains considerable doubt, however, as to whether either this trichomonad, or a rhizopod *Endamoeba gingivalis* found associated with diseased conditions of

the oral region by Kofoid (1929) and Wantland, Nakada, and Engel (1950), is to be considered as an etiological agent in these disorders, for they are also found in healthy mouths. Among domestic animals, *Trichomonas foetus* is definitely the causative organism of a venereal disease in cattle. It attacks the membranes of the vagina and uterus, causing abortions and delayed conceptions. *Trichomonas gallinarum* attacks the lower digestive tract of galliform birds and is often fatal in turkeys. *Trichomonas gallinae* is extremely injurious to the mucous membranes of the throat regions of pigeons and other birds, and sometimes invades the ducts of the liver and pancreas of these hosts.

While definite pathogenicity has been established in the case of only a few trichomonad flagellates, it would nevertheless seem prudent, until further evidence indicates otherwise, to (1) consider possible effects of the presence of these parasites in hamsters in the interpretation and evaluation of data from the many and varied experiments on this rodent, and (2) exercise precautionary measures against possible infection of laboratory workers by these protozoa.

Examination of hamsters in this study showed a rather high incidence of *Endamoeba muris* in the cecum (33.0 per cent). No intensive search has been made of the hamsters' mouths (teeth, periodontal tissues, and cheek pouches) for protozoan parasites. This needs to be done, as the presence of mouth amebas or flagellates might conceivably affect the caries and periodontal disease pictures in these animals. The original work by Arnold (1942) in experimental studies of caries has stimulated much research on the molar teeth as well as other studies of this animal. *Endamoeba muris,* so far as the author can determine, has not been previously reported from the hamster. This protozoan, reported from rats by many investigators, occurs in the hamster in both (1) the trophozoite stage (18 μ to 24 μ in diameter) with slow-moving pseudopodia, an eccentric karyosome in a chromatin-poor nucleus, and often showing ingested flagellates and erythrocytes in the cytoplasm, and (2) the cystic stage (15 μ to 20 μ in diameter) with from 5 to 8 nuclei. Evidence indicates that this protozoan is not pathogenic in either the hamster, the rat, or the human being. The physiologic activity and details of nuclear structure and nuclear division of trophozoites of this form from the rat have been rather completely described by Wenrich (1940). *Endamoeba histolytica,* a definitely pathogenic protozoan causing an often fatal diarrhea and dysentery in man, apparently does not occur naturally in the hamster, and according to experiments performed by Dr. G. Robert Coatney of the National Microbiological Institute (unpublished data), the hamster is refractory to experimental attempts of infection with this parasite.

Flagellate forms resembling *Giardia muris* and *Chilomastix bettencourti* were reported from one hamster by Wenrich (1946). Examination of 412 hamsters by the author revealed 9.22 + per cent infection with *Chilomastix bettencourti* (cecum) and 12.37 + per cent infection with a form resembling *Giardia muris* from the duodenum. The latter flagellate in some preparations also resemble *Giardia simoni,* which is claimed by Potter (1927) to be morphologically identical with *Giardia lamblia* from the small intestine of man. The giardias found in hamsters and reported in this paper show the characteristic shape of

body, resembling the outline of a spinning top (8 μ to 15 μ long). Eight flagella are present, all directed posteriorly. Two nuclei with large central endosomes are clearly evident, as are 2 slender axostyles. Some specimens show 1, others 2, deep-staining club-shaped parabasal bodies. In lateral view, the sucking disc can be readily seen. Oval cysts measuring 6 μ to 12 μ in length and containing from 2 to 4 nuclei are easily demonstrated.

The pathogenicity of giardias needs further study. Chandler (1952) stated that there is no longer any doubt of the pathogenicity of *Giardia Lamblia,* and Hegner (1924 and 1927) and Breuer (1938) found that this organism in heavy infections not only causes a flagellate diarrhea, but also may give rise to cholecystitis in man. Usually, however, giardias in man and other animals do not produce symptoms, but do multiply by binary fission and occasionally by multiple fission, and form cysts in enormous numbers which are passed by the host and are responsible for the initiation of new infections. Definite proof of a diarrhea induced by giardias is lacking, and it may be that large numbers of active giardias observed in certain diarrheas are the result of, rather than the cause of, this condition. It is just possible that the diarrheic condition from other causes provides a favorable environment for growth and multiplication of these flagellates. On the other hand, large numbers of giardias, adhering by means of their sucking discs to the wall of the small intestine, could conceivably interfere with absorption of fats, and large amounts of unabsorbed fats in the intestine do give rise to diarrhea.

Chilomastix bettencourti was found in the cecum of 9.22 + per cent of the hamsters examined in this study. Based upon evidence thus far, it is not to be considered a serious parasite. It resembles *Chilomastix mesnili,* a similar flagellate found in the large intestine of man, for which there is little evidence of pathogenicity. Westphal (1939), in experiments on himself, found *Chilomastix mesnili* to fluctuate with the condition of the intestine and feels that the presence of this protozoan in large numbers is a result of rather than the cause of intestinal disorders with which it may be associated. The trophozoite stage of *Chilomastix bettencourti,* as found in hamsters, is an unsymmetrical, pear-shaped form with a short, sharply pointed tail. It measures from 10 μ to 15 μ in length, and in wet preparations shows far less elasticity and variability in body shape than do trichomonads. There are 3 anterior flagella which continuously lash forward and backward, and in a relatively large cytostome there is a fourth flagellum attached to a short, undulating membrane which functions as a sort of "tongue" in securing particles of food. A round nucleus is present in the anterior part of the body. Unlike the continuous wobbly movements of trichomonads, *Chilomastix* forms exhibit a jerky spiral movement. Multiplication occurs by both binary and multiple fission. Lemon-shaped cysts are easily demonstrated, measuring from 6 μ to 9 μ in length, and in stained preparations the rounded nucleus, rich in chromatin, and the short, undulating membrane in the cytostome can be seen.

No representatives of the classes Infusoria or Sporozoa were found in this study. Forms resembling cystic stages of the ciliated *Balantidium coli,* in which

the organism can often be seen rotating by means of ciliary movements inside the cyst wall, were later found to be stages in the transformation of active trichomonads to the cystic form in which rotating motion was observed, due to wavelike movements of the undulating membrane around the periphery of the organism. Reports of hamsters either naturally or experimentally infected with protozoan ciliates do not appear to have been published.

Other than Toxoplasma (probably sporozoan) mentioned earlier, the only sporozoan that has been reported in the hamster is *Plasmodium berghei,* which has been used in experimental infections by Adler, Yoeli, and Zuckerman (1950) and Durand and Mathis (1951). This sporozoan, originally from Thamnomys, a tree-rat, causes fatal infections in hamsters in from 8 to 29 days, with as many as 50 per cent of the erythrocytes invaded by the parasite. It would be interesting to determine the susceptibility of hamsters to infection, with representatives of the Babesiidae, the Coccidia, Hepatozoon parasites, Hemogregarines, the Bartonella organisms, and the Rickettsias. Many of these give rise to serious disorders in other animals (rats, mice, rabbits, cats, dogs, hogs, and cattle), including fever, gastrointestinal disturbances, anemia, hemoglobinuria, and leukopenia. Much work remains to be done on the effect on the hamster of various protozoan infections.

In the study reported here, hamsters 2 years of age and over showed fewer intestinal protozoa than occurred in young animals, and no significant differences with regard to parasitism of male and female hamsters were noted. In further observations, the evidence thus far indicates that the intestinal protozoa normally found in the hamster are acquired sometime between the fourth and eleventh days following birth. Ratcliffe (1929) reported that removing young rats at 17 days of age from the mother and keeping them free from contamination, resulted in a protozoa-free colony of rats. Such a procedure would not be successful with hamsters since they acquire intestinal protozoa much earlier. Moreover, removal of the young at such an early date would in all probability result in exceptionally small animals and an unhealthy colony.

Various procedures have been employed in attempts to remove intestinal protozoa from experimental rodents. Hegner (1924, 1937) stated that *Endamoeba muris* is eliminated from rats, and other protozoa reduced in numbers, by feeding a high-protein diet. Ratcliffe (1928) concluded that the number of trichomonads in rats fed a high-protein diet was inversely proportional to the abundance of proteolytic anaerobic bacteria which were stimulated by protein. Nelson and Tatum (1938) stated that chiniofon acts as a well-tolerated trichomonicidal drug in rats and that the concentrations employed of such pentavalent arsenicals as carbarsone, mapharsen, acetarsone, and tryparsamide to kill trichomonads in vitro were many times the concentrations required in vivo. Gabaldon (1936) reported that 150 mg. of carbarsone per kilogram of body weight administered orally to rats each day for 5 days eliminated trichomonads. The author has successfully removed all intestinal protozoa from hamsters by feeding, for 7 days, a high-protein diet consisting of 45 per cent

ground beef liver, 42 per cent ground beef muscle, 11 per cent lard, and 2 per cent calcium carbonate. Animals fed this diet for as long as 3 months have shown slight gains in weight and appear to be in excellent condition. Considerable reduction in numbers, but not complete elimination of trichomonads and other protozoa from the intestine of hamsters, was achieved by administering carbarsone per os (15.6 mg. per 100 gram animal per day for 21 days). The feeding of the high-protein diet seems to be the simplest, safest, and most efficacious method of eliminating these parasites from the hamster.

HELMINTHES IN THE GOLDEN HAMSTER

The participation of large numbers of military and civilian personnel in World War II necessitated their living in endemic areas where there were firmly established tropical and oriental diseases which seldom, or never, had occurred in the United States. When these persons returned to their homes, many provided free nutrition, shelter, and transportation for parasitic forms foreign to this land. Among these parasites were the schistosomes or blood flukes. These trematodes spend a part of their life cycle in certain species of snails which serve as intermediate hosts. Cercariae developed within the body of the snail ultimately escape and penetrate the skin of man and other animals when the latter swim in, or wade through, cercaria-infested water.

With the recognition of cases of schistosomiasis in our returning service personnel, intensive studies were begun to determine whether any of the species of snails found in the United States could successfully play the role of intermediate host to blood flukes. The seriousness of the disease schistosomiasis is emphasized when it is remembered that a rather large percentage (5 per cent) of infections with *Schistosoma haematobium,* often termed "bilharziasis," were shown early by Virchow (1888), and subsequently by many others, to be associated with cancers of the bladder or rectum. Cram, Jones, and Wright (1945) demonstrated that a snail found in the southern United States, *Tropicorbis havanensis,* could transmit *S. mansoni,* the causative agent of human schistosomiasis in South America and the West Indies. Stunkard (1946), using snails that were descendants of the colony from the laboratory of Cram, Jones, and Wright, was unable to confirm their findings. He did, however, succeed in infecting hamsters, mice, rabbits, and monkeys with cercariae developed in *Australorbis glabratus,* a snail commonly found in South America and the West Indies, which was easy to raise in his laboratory. From cercariae developed in these snails, a strain of *S. mansoni* was developed in mice and hamsters which was carried through 10 generations. Attempts to establish rodent strains of *Schistosoma haematobium* and *Schistosoma japonicum* were unsuccessful. Brumpt (1930) reported 6 generations of *S. mansoni* in experimentally infected mice using the snail *Planorbis boissyi* as the intermediate host, and (1928) claimed to have achieved success in completing the cycle of *S. haematobium* from egg to egg in lower animals, recovering the eggs from the liver of a mouse. According to Stunkard's work, miracidia of the human schistosomes did not complete their development in representatives of the more common and readily available snails

from eastern United States. Schistosomiasis occurring naturally in the hamster seems not to have been reported and is not likely to occur, due to the habits and type of abode of these animals in nature.

Fairly successful treatment of cases of schistosomiasis has been achieved with intravenous injections of sodium antimony tartrate and other trivalent antimony compounds over a period of from 4 to 5 weeks. However, in advanced cases involving damage to the rectum or urinary organs, and in all cases of splenomegaly, surgery is necessary.

Few of the Cestoidea or tapeworms have been recorded from the golden hamster. Stunkard (1945) apparently was the first observer to record *Hymenolepis nana*, the so-called dwarf tapeworm, from the hamster. This tiny worm ranges from 7 to 100 mm. in length (usually 20 to 30 mm.), with a maximum breadth of 500 to 600 μ. According to Chandler (1952), this form is the most commonly found tapeworm in human beings in southern United States. Stunkard pointed out the dangers to laboratory workers and animal attendants of *H. nana* infections in experimental animals. Watson (1946) emphasized the danger of human beings acquiring helminth infections from handling hamsters. A total of 160 animals autopsied in his laboratory revealed 19 harboring *H. nana*. One animal yielded 22 worms. In experimental infections of mice and hamsters with *H. nana*, Larsh (1946) found that hamsters showed a greater percentage of development of adult worms, and their length at 11 days was greater than those in mice. Both the prepatent and patent periods were shorter in hamsters than in mice. Read (1951) experimentally infected hamsters with a strain of *Hymenolepis diminuta*, a much larger worm than *H. nana*, measuring from 30 to 60 cm. in length and 3 to 4 mm. in breadth, and possessing an unarmed scolex. The strain had been maintained in albino rats at the Rice Institute for 14 years. Natural infections were found in hamsters obtained from a commercial source in the Los Angeles area. The Los Angeles strain of worms was easily established in albino rats. Little or no host specificity of different strains of this tapeworm is indicated so far as rats and hamsters are concerned, according to these observations. It is important to keep in mind the fact that *H. diminuta* unlike *H. nana* requires an intermediate host for the development of cysticercoids. Grain-infesting insects, earwigs, dung beetles, fleas, and myriapods serve as intermediate hosts for this tapeworm. Human infections usually result from ingesting dried fruits and breakfast cereals contaminated with grain insects, which have become infected from rodent feces. *H. nana* was found by the writer in only 3 animals (0.72 + per cent) of 412 hamsters examined at autopsy. These 3 hamsters harbored 8, 11, and 18 worms, respectively. Animal attendants of the hamster colonies in which these worms were detected were advised to exercise diligence in washing their hands after handling animals or cleaning cages and encouraged to cultivate the habit of not placing fingers or instruments in their mouths. In this connection, one of the most dangerous habits observed by the writer in laboratory workers over a period of years is that of moistening labels or stamps with the tongue or lips. Many types of infections can result from such an unsanitary practice.

Animals in which Hymenolepis tapeworms have been detected should be isolated and insecticides employed to rid the animals and their quarters of insects, particularly grain-infesting forms and fleas. Hymenolepis infections can be effectively treated with several anthelminthic drugs. Filix mas, or male fern, carbon tetrachloride, and hexylresorcinol have been extensively used, and gentian violet has proved especially effective against *H. nana.*

While studying the sarcoma-producing power in white rats of *Cysticercus fasciolaris,* the larval form of the cat tapeworm *Taenia taeniaformis,* Wantland (1953) was unsuccessful in experimental attempts to establish this cysticercus in the liver of hamsters. However, one unexplained infection with a single *C. fasciolaris* strobilocercus was found at autopsy in the liver of a hamster from a separate series which had been experimentally infected with *Trichinella spiralis.* Among the possibilities to account for the infection are (1) the chance ingestion of an extremely viable egg, or (2) some peculiar phenomena provided by the trichinae or provoked by the concomitant presence of the 2 parasites which was conducive to invasion of the liver by an active oncosphere released after accidental ingestion. Earlier experimental work on trichinosis in rodents by the writer (Wantland, 1934, 1935) stimulated the maintenance of *T. spiralis* in rabbits, rats, and hamsters for some years for other studies of this parasite (1937, 1938, 1945, 1947). The course of trichinosis in the hamster is much the same as in other animals except that (1) the hamster shows a much lower tolerance for from 1,000 to 2,000 larvae fed (intestinal and muscle phases of disease) than do rats, rabbits, or guinea pigs, and (2) while the histologic picture of the normal encystment and encapsulation processes is similar to that occurring in other rodents, acceleration of calcification of cysts through administration of irradiated ergosterol and calcium lactate is not nearly so marked in the hamster. Humes and Akers (1952) stated that in hamsters experimentally infected with *T. spiralis,* the larvae in the striated muscle fibers of the cheek pouch become surrounded very early (nineteenth day after infection) by a rich, vascular network of venous nature. This increased vascular supply remained up to the 280th day of infection, and then gradually diminished until it became inconspicuous on the 341st day following infection. Early in the infection (3 days), leukocytes appeared sluggish in veins and venules of the cheek pouch, and mural thrombi occurred in these vessels between the sixth and eleventh days of infection.

Lentze (1935) claimed that ova of the human pinworm, *Enterobius vermicularis,* were liberated into the air when sheets and clothing contaminated with them were shaken, and he suggested that the eggs might be inhaled to produce infection. In studies by Noland and Reardon (1939), Schüffner and Swellengrebel (1944), and Wantland, Ho, Carmichael, and Storm (1950), pinworm ova were found in dust at all levels of rooms in various public and private buildings examined, on the floor, upholstery, toilet seats, light fixtures, and on the molding of doors and windows. The results of these studies indicate that the possibility of infection and reinfection by inhalation of viable pinworm ova must be considered in the prophylaxis and treatment of pinworm infections.

The oxyurids of various mammals, as shown by Seurat (1916), have life cycles similar to *E. vermicularis*. So far as is known, infection is always direct without involvement of an intermediate host. Watson (1946) reported *Syphacia obvelata* from the cecum and colon of 83 out of 160 hamsters examined. This roundworm was found in only 2 of the 412 hamsters examined by the writer. The male of this species measures from 1.5 to 2.0 mm. in length and the female from 3.0 to 6.0 mm. in length. The eggs are thin-shelled, flattened on one side, and measure 110 to 140 μ by 30 to 40 μ. *S. obvelata* has been reported from the human being by Riley (1919). The presence of oxyurid worms usually causes an intense anal pruritus in the host due to periodic, generally nocturnal, migrations of the female worm out the anus, where she deposits eggs in the perianal region. Chan (1951), in studying the life cycle of *S. obvelata* in mice, confirmed direct infection of one animal to another by eggs and stated that bacitracin in large doses, given for two consecutive days, removed all larvae and young adults, and caused premature migration of female worms. In chemotherapeutic studies of pinworm infections of mice, Reinertson and Thompson (1951) found gentian violet, crystal violet, phenothiazine, hexylresorcinol, and tetrachlorethylene all effective against *S. obvelata*. These same drugs are used with success in the treatment of human cases of Oxyuriasis.

The story of Fibiger's work on cancer of the stomach of rats, employing *Gongylonema neoplasticum* as a parasitic inciting agent (Oberling, 1944), is a scientific Odyssey. Fibiger (1913, 1920), employing Borel's hypothesis (1907, 1909) of the parasitic origin of cancer, reported, after many discouraging experiences, papillomas and carcinomas in the stomachs of rats infected with *G. neoplasticum*. Fibiger definitely proved that cockroaches act as intermediate hosts for this nematode. Of interest in this connection is the report of Mauer, Stauber, and Grun (1953) on the finding of a related form, *Protospirura muris*, in the eosophagus and stomach of the hamster. This worm is also transmitted from host to host by the eating of cockroaches and, in the definitive host, blocks the esophagus and irritates the walls of the stomach, sometimes causing perforations. The susceptibility of the hamster to this parasite suggests the use of the latter in carcinogenic experiments on hamsters, and because of the position of the worm in the anterior parts of the ailmentary tract, valuable screening tests of compounds for chemotherapeutic activity might be readily accomplished without loss due to absorption and excretion of the compound by the host.

ARTHROPOD PARASITES OF THE HAMSTER

The golden hamster apparently plays natural host to comparatively few arthropods, either as endoparasites or as ectoparasites. The amazingly clean habits and practices of this animal may account, in large part, for the absence of forms which not uncommonly infest the surfaces of bodies, especially heavily "forested areas," as well as certain readily accessible cavities, of many hosts

higher in the taxonomic scale (including *Homo sapiens*) than the fastidious hamster. Nocturnal in natural habitat, the hamster might seem to provide a good target for certain insect vectors which are known to swarm forth in greatest numbers at night on their deadly mission of transmitting disease. However, the extreme alertness and almost constant activity of the hamster, plus the fact that few parts of the skin are devoid of thick hair, would certainly present a problem to even the most persistent insect or most tenacious arachnid.

Reports in the literature of either accidental or planned experimental infection or infestation of the hamster with parasitic members of the class Arthropoda are few. Florio, Stewart, and Mugrave (1944) employed hamsters in experimental studies on the transmission of Colorado tick fever, a disease caused by a filterable virus, carried by a tick *Dermacentor andersoni*. These workers transmitted this disease serially in human beings and hamsters by parenteral injections of blood and serum, but were unable to demonstrate transovarial transmission in *D. andersoni*. The disease is in many ways similar to dengue fever except that the latter is transmitted chiefly by mosquitoes of the Stegomyia group. An initial high fever, severe prostration, and leukopenia are characteristic and relapses are not infrequent. The infection is not fatal in man or experimental animals but convalescence is slow. *D. andersoni*, in its immature stages (six-legged larvae 0.5 to 1.0 mm. long and eight-legged nymphs 1.3 to 1.5 mm. long), acts as a parasite of rabbits and certain rodents, especially squirrels, in the western United States. After dropping off these hosts and transforming into adult ticks (3.0 to 6.0 mm. long), they tend to seek larger animals, such as horses and cattle, although they readily attach to other animals as well as man. There is no evidence that ticks occur regularly or even occasionally as ectoparasites of the hamster in nature.

Contrary to the writer's experience, Worden (1949) claims that Notoedric mange, caused by a minute burrowing mite, *Notoedres notoedres*, is quite common in the hamster. In this disease, the infection may spread to all parts of the body, and sections of the skin reveal eggs, larvae, nymphs, and adult mites. Adult males measure from 0.1 to 0.2 mm. long and females from 0.3 to 0.4 mm. long. Eggs are laid by the female mites at the ends of the burrows, and hatch in from 3 to 4 days into six-legged larvae. The larvae transform into eight-legged nymphs in about 3 days. Nymphs burrow into the skin and moult, probably twice, becoming adult mites. The whole cycle takes from 6 to 10 days. Infection takes place by direct contact of one animal with another. Mites of the genus *Notoedres* do not seem to establish themselves in the skin of man as does *Sarcoptes scabiei*, but may remain on human beings for short periods of time. Fulton (1943), in treating *Notoedres* infections in hamsters, found 2 organic sulfur compounds, dimethyl diphenylene disulfide (Mitigal) and tetraethylthiuram monosulfide, highly effective. Either of these compounds can be procured in a 10 per cent emulsion in commercial coconut oil. Treatment by rubbing affected areas with the emulsion once every 2 or 3 days until 4 treatments have been administered usually results in a cure.

TABLE II
INCIDENCE OF PARASITES IN 412 HAMSTERS FROM 8 SOURCES*

SOURCES	NUMBER HAMSTERS EXAMINED	TRICHOMONADS (SEVERAL SPECIES)		ENDAMOEBA MURIS		GIARDIA (MURIS?)		CHILOMASTIX BETTENCOURTI		HYMENOLEPIS NANA		CYSTICERCUS FASCIOLARIS		SYPHACIA OBVELATA		MUSCA DOMESTICA (LARVAE)	
		NO. INF.	PER CENT	NO. INF.	PER CENT	NO. INF.	PER CENT	NO. INF.	PER CENT	NO. INF.	PER CENT	NO. INF.	PER CENT	NO. INF.	PER CENT	NO. INF.	PER CENT
Univ. of Rochester	154	154	100.0	51	33.1	17	11.0	6	3.9								
Illinois Wesleyan University	180	179	99.4	51	28.3	29	16.1	26	14.4	2	1.11	1	0.55	2	1.11	1	0.55
Harvard U.	13	11	84.6	9	69.2												
Walter Reed Hospital	4	4	100.0	2	50.0					1	25.0						
Univ. of Alabama	17	17	100.0	6	35.3	1	5.9										
Univ. of Minnesota	14	13	92.8	7	50.0	1	7.1										
Gulf Shores Hamstery	14	14	100.0	8	57.1	3	21.4	4	28.6								
Gen. Biol. Supply House	16	16	100.0	2	12.5			2	12.5								
Total	412	408	99.27	136	33.0	51	12.37	38	9.22	3	0.72	1	0.24	2	0.48	1	0.24

*August 6, 1955.—Since this report was submitted for publication further examination of material collected during the original study plus a study of 88 more hamsters from the Illinois Wesleyan University colony revealed the following additional parasites (incidence given in percentages based on total of 500 animals): *Hexamita muris*, 3.2 per cent; *Hexamita pulcher*, 2.4 per cent; *Enteromonas hominis*, 1.0 per cent.

Man and other animals are known to have been subjected to attack for centuries by numerous types of adult flies and by maggots or the larvae of many species of flies. The common housefly, *Musca domestica,* only fairly recently has been subdued with modern screening of buildings and highly effective insecticides. Intestinal myiasis was observed by the writer in a hamster that had appeared sluggish and lethargic for 2 or 3 days preceding death. Autopsy revealed 19 living *M. domestica* larvae, 10 to 13 mm. long, established in the rectum, causing complete stasis, intestinal hemorrhage, and marked flatulence. Experimental attempts to infect other hamsters by placing them together with flies in fly-proof containers were unsuccessful. Further experimentation is planned to determine the method of infection of the hamster by this fly larva. The infection here mentioned might have originated in either of 2 ways: (1) the hamster ingested food contaminated by fly eggs or larvae, and the larvae succeeded in reaching the rectum alive; or (2) fly eggs were deposited in close proximity or association with the hamster, and the larvae entered the anus.

The common bedbug, *Cimex lectularius,* has been found at times in varying numbers in animal quarters by many experimental investigators. *C. lectularius* has been noticed in the animal quarters of this laboratory only twice. In both instances a single bug was observed in cracks of wooden cages housing hamsters. Further evidence is needed before reporting this insect as an ectoparasite of the hamster. The newer insecticides, DDT, chlordane, and benzene hexachloride, are all highly effective in getting rid of bedbugs. These substances, when applied in the form of dusting powders or emulsion sprays into all joints and crevices of cages and cracks between floor boards, will eliminate this nocturnal prowler in 2 or 3 days.

Another night prowler with a voracious appetite for many foods and observed singly upon occasion during the day around animal quarters is the American cockroach, *Periplaneta americana.* This insect varies in color from light brown to almost black and adults measure from 30 to 40 mm. in length. Although no evidence has been presented to date that *P. americana* acts either as an ectoparasite of the hamster or as a vector of diseases due to animal parasites of this rodent, knowledge of its role (Fibiger, 1913) as an intermediate host of *Gongylonema neoplasticum,* the nematode giving rise to carcinoma of the stomach wall in rats, should be sufficient reason for the maintenance of a high level of interest in research workers in the control, if not the eradication, of this unsanitary pest.

Well-lighted, clean, and neatly kept quarters are not conducive to the development of large numbers of cockroaches. One of the best insecticides for roach control is a dusting powder mix consisting of 25 per cent by volume of sodium fluoride and 75 per cent tested pyrethrum powder. Application of this mix in the evening at intervals of 1 or 2 weeks, to cracks, crevices, along baseboards of walls, and around food purposely set out as bait will soon result in fatal poisoning of these pests.

In working with hamsters, the writer has frequently noticed and collected specimens of a species of Collembola. This "springtail" arthropod is found both on droppings below the cages and on the bodies of hamsters. Rubbing the fur back and forth on animals removed from cages has not infrequently disturbed these minute wingless insects (1 to 3 mm. in length), causing them to spring from the host, landing on a laboratory table where they continue to spring by means of the springing organ (furcula), especially under strong illumination. The species of this Collembolan has been determined as *Willowsia platani* Nicolet f. *nigromaculata* Lubbock.* It is possible that these forms are only temporary "riders" and accidentally arrive in the fur of the hamster from the pans below the cages in precisely the same manner that they disembark, i.e., by springing upward. Further investigation is certainly indicated, for even though these Collembola may spend only short periods of time on the bodies of hamsters and not exercise their chewing mouth parts, they, like the bedbug and cockroach, must be viewed with suspicion since they may act as the intermediate host of some parasite, or carry on their legs or body parts infective stages of parasitic protozoa or worms which could be ingested along with the insect when captured by the definitive host.

Parasitic forms of protozoa, helminthes, and arthropods found by the writer in examinations of 412 golden hamsters from 8 sources are indicated in Table II.

SUMMARY

1. The origin of the golden hamster, *Cricetus auratus* (Waterhouse), as an experimental laboratory animal is reviewed.

2. The growing popularity of the hamster as an experimental animal in many areas of biologic and medical research has resulted in (1) increased breeding of this animal in both commercial hamsteries and experimental laboratories, and (2) a definite need for further information on this rodent with reference to its anatomy, physiology, patterns of behavior, parasitic fauna and flora, nutrition requirements, and measures of prevention and control of disease.

3. The following animal parasites reported from the hamster by various investigators are described, life cycles reviewed, physiologic activity and pathogenicity discussed, and methods of prevention and control indicated: Toxoplasma (sp?), *Leishmanis donovani, Leptospira icterohaemorrhagiae, Leptospira canicula, Trichomonas muris, Trichomonas wenyoni, Trichomonas minuta, Trichomonas microti,* a fifth type of trichomonad not previously described, *Giardia (muris?), Chilomastix bettencourti, Endamoeba muris, Plasmodium berghei, Schistosoma mansoni, Hymenolepis nana, Hymenolepis diminuta, Cysticercus fasciolaris, Syphacia obvelata, Protospirura muris, Notoedres notoedres,* and *Musca domestica.* Found in association with hamsters and highly

*The author is indebted to Dr. Elliott A. Maynard, Department of Pharmacology and Toxicology, School of Medicine and Dentistry, University of Rochester, for identification of this Collembolan.

suspected but still of questionable role as ectoparasites or vectors in relation to these animals are reported: *Cimex lectularius, Periplaneta americana*, and a species of Collembola.

4. The incidence of parasitism in a recent study of 412 hamsters examined from eight sources is presented in tabular form (Table II).

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CHEMICAL AND X-RAY EXAMINATION OF DEPOSITS REMOVED FROM THE TEETH OF GOLDEN HAMSTERS AND FERRETS

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IN 1944, King[20] observed the formation of deposits on the teeth and the occurrence of periodontal disease in ferrets, closely resembling that disease in man, which is associated with calculus formation and food impaction. The material deposited on teeth was found to resemble human salivary calculus both clinically and histologically.

King and Gimson,[23] attempting to repeat the production of experimental caries in the molar teeth of the golden hamster, as reported by Arnold[1] and later by other workers,[6, 19, 29] observed that when these animals were fed a high-cereal diet, a form of periodontal disease occurred, associated with food impaction and the presence of deposits on the teeth histologically similar to human salivary calculus. King[21] later observed similar lesions in hamsters fed a diet composed principally of raw or refined sugar. A varying proportion of the teeth also showed cavities, some of which, in an advanced stage, resembled those due to human caries, although it was noted that such cavities most frequently arose in the cementum.

An indication of the calciferous nature of the deposits was obtained when scrapings from the teeth of both species were found to give a positive reaction to Lillie's[27] modification of the alizarin red S staining technique of Langeron.[26] A more complete chemical examination was then undertaken, together with x-ray examination, to compare the composition and crystal structure of the deposits with those of human dental calculus.

MATERIALS AND METHODS

Diet.—Hamsters were maintained from weaning, at the age of 6 weeks, on one of the diets shown in Table I. Some groups of animals were fed these diets with the addition of small concentrations of supplements, as follows: sodium fluoride at a level of 50 parts F′ per million; sodium iodoacetate at a level of 200 parts CH_2ICOO' per million; sodium thioglycollate at a level of 100 parts CH_2SHCH_2COO' per million.

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Ferrets were maintained from weaning, at the age of 7 to 8 weeks, on the diet shown in Table II.

TABLE I
HAMSTER DIETS

	PER CENT BY WEIGHT
(a) Cereal	
White bread (crumb)	67
Whole milk powder	7.4
Alfalfa	14.8
Brewer's yeast	3.7
Sodium chloride	3.7
Peanut oil (+ vitamins A and D)*	3.7
(b) Sugar	
Refined sugar (sucrose)	63
Casein	21.4
Alfalfa	4.9
Brewer's yeast	4.9
Sodium chloride	1.0
Peanut oil (+ vitamins A and D)*	4.9
Animals received 10 Gm. daily of (a) or (b) and, in addition, 5 Gm. fresh cabbage twice a week, and water ad libidum.	
(c) Cubed (as given in[5])	
Wholemeal flour	45
Ground oats	40
Fish meal	8
Dried yeast	1
Dried skimmed milk	3
Cod liver oil	1
Sodium chloride	1
Animals received the diet ad libidum and also 5 Gm. fresh cabbage twice weekly, and water ad libidum.	

*Peanut oil contained 1,000 I.U. vitamin A, and 100 I.U. vitamin D per milliliter.

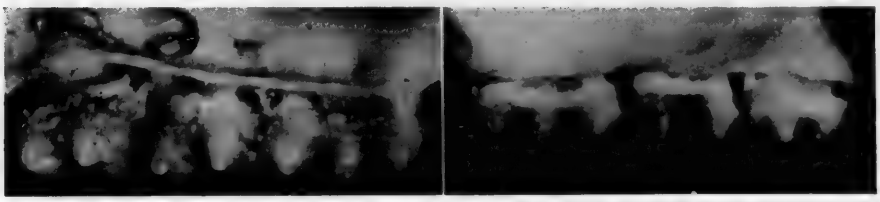
TABLE II
FERRET DIET

Amounts of diet fed per diem to each animal:	
White bread (crumb)	60 Gm.
Meat (lean)	10 Gm.
Whole milk	114 ml.
Food yeast	0.85 Gm.
Salt mixture (McCollum 185)	1.0 Gm.
Peanut oil	2.0 ml.
Containing vitamin A	2,000 I.U.
Containing vitamin D	200 I.U.

Preparation of Material.—The deposits on hamster and ferret teeth were exposed by autoclaving the jaws of the animals, excised shortly after death, under water, for 15 minutes at 15 pounds per square inch. The soft tissue was then washed away under a jet of cold water, and the teeth and jaws dried in vacuo over anhydrous calcium chloride. The appearance of the material in situ is shown in Figs. 1 and 2. The teeth were extracted from the jaws and the deposits removed from the teeth with dental scalers. The physical resemblance to human calculus was evident here in the hardness of the material,

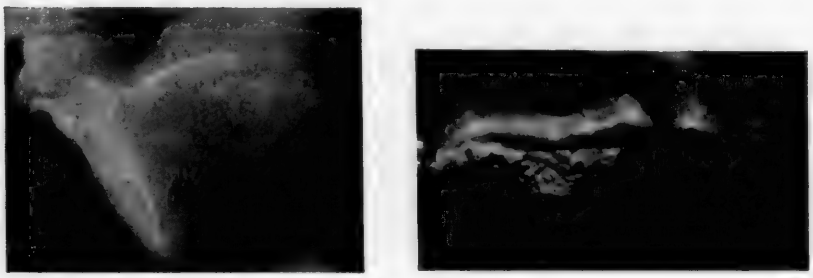
Human calculus was collected in saline solution directly from the mouths of patients and from freshly extracted teeth, washed briefly in distilled water, and dried in vacuo over anhydrous calcium chloride. For some of the x-ray examinations parallel-sided ground sections, approximately 0.2 mm. in thickness, were prepared from whole teeth with calculus in situ.

Chemical Analysis.—Samples of the dried materials (0.1 to 10 mg.) were weighed into 15 ml. centrifuge tubes and extracted with 5 ml. N hydrochloric acid at room temperature for 12 hours, with occasional stirring. The insoluble matter was then centrifuged down and aliquots of the supernatant solution withdrawn for analysis.



A. *B.*

Fig. 1.—*A*, Slight, and *B*, heavy deposits on the molar teeth of autoclaved upper jaws of the hamster (×10; reduced ⅙).



A. *B.*

Fig. 2.—*A*, Slight deposit on the upper PM_4 tooth of an autoclaved ferret jaw (×10; reduced ⅙); *B*, heavy deposit of upper PM_3 and PM_4 teeth of an autoclaved ferret jaw (×7; reduced ⅙).

Calcium was precipitated as oxalate by the Clark-Collip technique, the precipitate separated by centrifugation, and washed twice with 2 per cent ammonium hydroxide saturated with calcium oxalate. The resulting precipitate was dissolved in N perchloric acid and titrated with 0.01 N ceric ammonium nitrate in N perchloric acid, using σ-phenanthroline ferrous sulfate as indicator. The ceric reagent was prepared fresh from a stable stock 0.1 N solution in 2 N sulfuric acid by dilution with perchloric acid.

Phosphorus, as orthophosphate, was estimated by the method of Berenblum and Chain,[3] with the use of separate flasks for the stannous chloride reduction, as recommended by Ennor and Stocken.[8]

X-ray Examination.—Both x-ray diffraction and microradiographic methods were employed. Most of the x-ray diffraction photographs were obtained from samples composed of a few milligrams of material, using a cylindrical camera of 19 cm. diameter. Some photographs were obtained from ground human tooth sections, using a transmission method with a flat film. By this method, areas of less than 1 mm. in diameter could be examined separately. Copper Kα radiation was employed throughout.

Each of the samples of material was then heated at 900° to 1,000° C. for one hour and re-examined. The diffraction photographs were compared visually with standards, e.g., from synthetic hydroxyapatite, and from hydroxyapatite prepared by heating bone or dental enamel at 900° to 1,000° C., all with the appropriate Ca:P ratio. From this comparison, it was possible to identify the predominant crystalline compounds present.

Microradiographs of some sections of human teeth, in which calculus was present, were obtained, using the characteristic radiation of copper. The techniques employed, like the diffraction techniques referred to previously, were similar to those already described by Thewlis.[35]

TABLE III

DIETARY SUPPLEMENTS AND DEPOSITS ON GOLDEN HAMSTER TEETH

DIET (26-WEEK PERIOD)	NO. OF ANIMALS	VISUAL GRADING PER TOOTH	WEIGHT DEPOSIT OBTAINED PER TOOTH ×10
Sugar, basal	11	1.9	3.5 mg.
Sugar + 50 ppm F′ as sodium fluoride	10	1.0	1.7 mg.
Sugar + 200 ppm iodoacetate (Na salt)	7	1.2	2.4 mg.
Sugar + 100 ppm thioglycollate (Na salt)	9	1.3	2.7 mg.

RESULTS

Hamster.—The amount of material found on the molar teeth of hamsters was generally small after a 26-week dietary period, but often considerable after longer periods. Qualitatively, the cereal diet appeared to produce greater deposits than the sugar diet. Animals fed a stock cubed diet[5] developed very little deposit, probably owing to the hardness of the diet, although calcification of a food impaction has been noted. The presence of small concentrations of fluoride or iodoacetate in the diet appears to reduce the formation of the deposit as estimated visually under a low-power microscope, or from the weight of material obtained on scaling the teeth (Table III).

The majority of specimens show contents of calcium and phosphorus comparable with those of human calculus (Table IV). While the percentages are

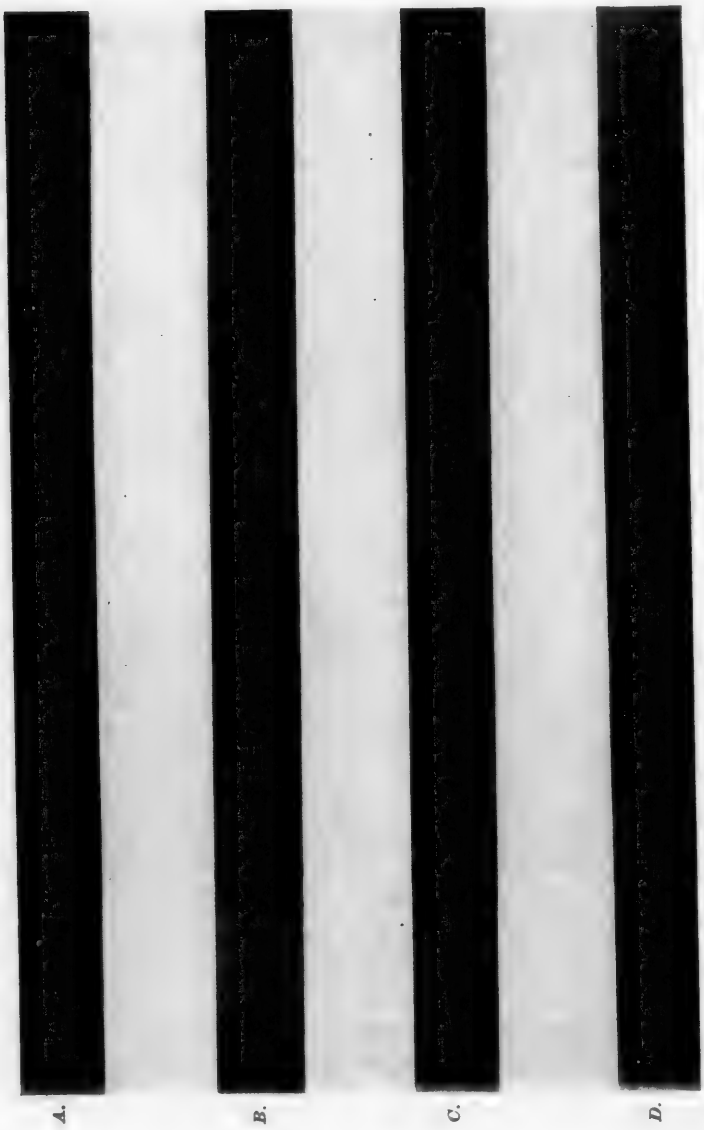


Fig. 3.—X-ray diffraction photographs given by specimens of the animal deposits. The photographs, while typical of those generally obtained, have been selected to illustrate the range of patterns observed. *A*, Unheated, showing hydroxyapatite patterns; *B*, after heating at 900° to 1,000° C. for one hour, showing hydroxyapatite pattern; *C*, after heating at 900° to 1,000° C. for one hour, showing hydroxyapatite and β-$Ca_3(PO_4)_2$ patterns; *D*, after heating at 900° to 1,000° C. for one hour, showing β-$Ca_3(PO_4)_2$ pattern.

generally lower, the calcium:phosphorus ratios fall in a range similar to those found in human calculus. There is no obvious difference in composition between the deposits obtained from animals fed cereal or sugar diets.

TABLE IV
CHEMICAL ANALYSES OF DEPOSITS FROM THE TEETH OF THE GOLDEN HAMSTER

DIET	EXPERIMENTAL PERIOD (WEEKS)	NO. OF ANIMALS	PER CENT Ca	PER CENT F	Ca:P
Cereal	ca. 52	6	20.3	9.8	2.07
Cereal	50-63	3	23.9	12.1	1.98
Cereal	49	1	26.5	11.4	1.75
Cereal	17	1	5.0	4.0	—
Cereal (+ 200 ppm iodoacetate)	25-49	3	19.5	10.5	1.86
Cereal (+ 50 ppm F′)	46	1	29.6	10.8	1.91
Sugar	26	12	24.5	11.0	2.23
Sugar	26	11	12.7	6.2	2.05
Sugar (+ 50 ppm F′)	26	10 Lower	32.2	15.5	2.08
		10 Upper	22.3	10.5	2.12
Sugar (+ 200 ppm iodoacetate)	26	7 Lower	19.7	8.9	2.21
		7 Upper	17.9	10.3	1.74
Sugar (+ 200 ppm iodoacetate)	26	4	4.0	2.0	—
Sugar (+ 100 ppm thioglycollate)	26	9 Lower	9.7	5.3	1.83
		9 Upper	21.4	11.3	1.89

Examination of the x-ray diffraction photographs of unheated specimens from hamster teeth showed that all gave a hydroxyapatite pattern which was indistinguishable by eye from that produced by bone or dentin (Table VI, Fig. 3). The photographs from heated specimens in all cases had sharper reflections than those obtained before heating, presumably owing to the aggregation of crystallites. In some heated specimens, lines attributed to β-$Ca_3(PO_4)_2$[2] were also observed.

TABLE V
CHEMICAL ANALYSES OF DEPOSITS FROM THE TEETH OF FERRETS

SPECIMEN	NO. OF ANIMALS	PER CENT Ca	PER CENT F	Ca:P
1. Pooled, from upper jaws after dietary period of 1 year	22	19.0	10.0	1.90
2. Pooled, lower jaws	22	18.2	10.3	1.77
3. Pooled, dietary period 1 year; upper jaws	4	20.6	10.05	2.05
4. Pooled, lower jaws	4	15.2	8.2	1.85
5. Pooled, dietary period 1 year with bone supplement for last 3 months; lower jaws	6	13.3	6.4	2.08
6. Pooled, dietary period about 3 years with various periods of bone supplements; lower jaws	2	17.85	8.2	2.17

TABLE VI

X-RAY DIFFRACTION PATTERNS OF DEPOSITS FROM THE TEETH OF THE GOLDEN HAMSTER AND THE FERRET

SPECIES	DIET	DIFFRACTION PATTERN	
		BEFORE HEATING	AFTER HEATING AT 900° TO 1,000° C. FOR 1 HR.
Hamster	Cereal basal	Hydroxyapatite	Hydroxyapatite + β-Ca_3 $(PO_4)_2$
Hamster	Cereal basal	Hydroxyapatite	Hydroxyapatite
Hamster	Sugar basal	Hydroxyapatite	—
Ferret (upper)	Cereal basal	Hydroxyapatite	Hydroxyapatite + β-Ca_3 $(PO_4)_2$
Ferret (lower)	Cereal basal	Hydroxyapatite	Hydroxyapatite + β-Ca_3 $(PO_4)_2$
Human (pooled lower incisor)		Hydroxyapatite	Hydroxyapatite

A.

B.

Fig. 4.—*A*, Microradiograph (×10) of ground section of human lower incisor tooth showing relative calcification of enamel (*E*), dentin (*D*), and two adjacent calculus deposits (C_1 and C_2).

B, Photomicrograph (×10) of the same area of the ground section.

Ferret.—The chemical analyses of these deposits also show similarity to those of human calculus (Table V), although, as with the hamster material, the calcium and phosphorus contents fall in a lower range.

The x-ray diffraction photographs of pooled ferret material taken from the upper and from the lower jaws both showed only the hydroxyapatite pattern when unheated, and hydroxyapatite and β-$Ca_3(PO_4)_2$ patterns after heating.

Human.—A pooled specimen obtained from the lower incisors gave an x-ray diffraction photograph similar to hydroxyapatite both before and after heating. Calculus in the ground sections gave a similar pattern before heating, while,

after heating, different regions produced a pattern similar either to hydroxyapatite, or to hydroxyapatite and β-$Ca_3(PO_4)_2$. A few regions gave lines of β-$Ca_3(PO_4)_2$ pattern before heating, but this was considered to be due to the effect of local heating during the preparation of the section.

Microradiographs of ground sections of human calculus showed considerable variation in mineral density extending from less than that of dentin to almost as much as that of enamel, and also the frequent occurrence of layers of calculus of differing appearance on adjacent portions of the same tooth. This is illustrated in Fig. 4, in which clearly defined zones can be seen, suggesting that the calculus has been formed in different ways. It is of interest to note that, in this example only, the outer zone showed, after heating, diffraction lines due to β-$Ca_3(PO_4)_2$.

The use of autoclaving in the preparation of the animal specimens might be expected to have some effect upon the composition of the deposit. In particular, such a procedure might result in an alteration in the degree of calcification (e.g., by loss of organic matter rendered water-soluble). The results of an experiment to compare the composition of the deposits on ferret teeth before and after autoclaving are not yet available, but the analyses of a limited number of comparable samples taken from ferret teeth in vivo are within the range of those reported here. It has also been observed that autoclaving ground human calculus (Ca, 28.0 per cent; P, 15.6 per cent, organic matter ca. 25 per cent), under the conditions described, produced no significant change in the calcium and phosphorus contents.

DISCUSSION

The deposits which have been found on the teeth of hamsters and ferrets are, in general, considerably calcified and show chemical and crystallographic similarity to human dental calculus, in addition to the clinical and histologic resemblance previously reported.

The occurrence of dental calculus in dogs, cats, and horses is well known. It has also been reported in mules and pigs,[37] and in certain wild animals, including the elephant,[10] the bear,[16] and the South African golden mole.[13] Under certain conditions, it has been observed in the laboratory rat.[24]

Chemical analyses indicate that the carbonate content of human calculus is low[12, 18, 25] and of horse calculus very high,[11, 31] while that of dog calculus shows an intermediate level.[31] Although no chemical analyses for CO_2 have been made on the hamster and ferret material, qualitative observations suggest that it is more similar to human than to dog or horse calculus in this respect.

The main difference between the animal calculus which was examined, and the human material, lies in the smaller degree of calcification found in the animal deposits. This may be related to the age of the deposit, the effect of which on the composition is being investigated. The selection of experimental material is probably an important factor. In the animal experiments, all of the available material has been used for analysis owing to the limited amounts present, whereas it is possible to select samples of human material according to clinical characteristics.

That variation in the extent of calcification of human calculus can occur is shown by chemical analyses.[12, 18, 25] This conclusion has been extended by radiographic examination to show that considerable differences exist between deposits on the same tooth.

The finding of a hydroxyapatite-like structure in all samples of material examined is in agreement with the results of several workers who have studied human dental calculus by x-ray methods.[7, 9, 17, 28, 30, 34] However, with one exception, the x-ray diffraction photographs given by unheated specimens do not show the presence of any structure other than hydroxyapatite. In the one exception, already referred to, there is a strong possibility that the result was an artifact of local heating during preparation of the section. These observations do not exclude the possibility of the presence in small amounts of other components.

After specimens are heated at 900° to 1,000° C., the inorganic portion of calculus either remains as hydroxyapatite or is changed into a mixture of hydroxyapatite and β-$Ca_3(PO_4)_2$. Again no evidence has been obtained of the presence of any other compound. Results so far obtained show that the β-$Ca_3(PO_4)_2$ may be obtained in heated specimens from all three species, while, in human calculus, the presence of different types of calculus on the same tooth has been demonstrated.

The reasons for the partial conversion on heating of the hydroxyapatite of calculus, and other calcified biologic material, into β-$Ca_3(PO_4)_2$ are not entirely clear. Trömel and Möller,[36] and later Hodge, Le Fevre, and Bale,[15] considered that the appearance of β-$Ca_3(PO_4)_2$ on heating synthetic tertiary calcium phosphates was due to the reaction between excess phosphate (adsorbed $CaHPO_4$ or phosphate ions) and the hydroxyapatite. Hirschman, Sobel, Kramer, and Fankuchen,[14] and Sobel, Hanok, Kirshner, and Fankuchen[32] came to a similar conclusion from studies of high phosphate bones from rats, and also showed that β-$Ca_3(PO_4)_2$ resulted when a synthetic mixture of $CaHPO_4$ and hydroxyapatite was heated at 900° C. Philipp[30] suggested that the presence of β-$Ca_3(PO_4)_2$ in heated human calculus was due to the formation of the calculus in a phosphate-rich medium (human saliva). Mathis,[28] in a sample of gray calculus, and recently Jensen and Danø,[17] in salivary calculi, claimed to have observed the presence of β-$Ca_3(PO_4)_2$ in unheated specimens. It is hoped that studies in progress will make the significance of these latter observations clear.

While it has been shown that some, at least, of the hamster deposits are calcified, the exact relationship between the cavitation of the teeth and the calcification of the organic matrix of the calculus remains to be investigated. Some years ago Berke[4] observed that, histologically, the organic matrix of human tartar was similar to what is commonly designated "bacterial plaque," and the difficulty of drawing conclusions from microscopic observations is still evident (cf. King,[22] who has suggested that the "bacterial plaque" might be the common precursor of both carious processes and calculus formation).

Stephan,[33] in a recent article, concluded that much of the controversy over the role of dental plaques and microorganisms in the caries process had been due to reading more into the microscopic picture than is shown by normal histologic techniques. The results reported here, especially in relation to the hamster, emphasize that interpretations made from histologic observations should be made with caution unless, or until, they can be supported by reliable histochemical studies.*

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EXPERIMENTAL DENTAL CARIES

VII. The Effect of Various Androgens and Estrogens on Dental Caries in the Rat

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PREVIOUS work in these laboratories has indicated that orchiectomy consistently reduces the dental caries incidence in rats, while ovariectomy has produced variable results.[1-3] The administration of testosterone to control males appeared to increase the dental caries incidence, while in orchiectomized rats, testosterone was without effect. Estrogens (estradiol and diethylstilbestrol) increased dental caries in the control male, control female, and ovariectomized rats. The androgens appeared to exhibit little effect on the dental caries experience in immature rats, while the estrogens appeared to increase the caries incidence. Furthermore, the increased caries effect produced by estrogen administration seemed more pronounced in gonadectomized rats than in unoperated animals.

In order to extend these observations, it was decided to compare another androgen (methylandrostenediol) with testosterone in both unoperated and gonadectomized animals. Also, since it had been indicated[2] that in one instance diethylstilbestrol reduced caries experience, it was thought necessary to attempt to clarify this point by repeating this portion of the work. This report will summarize the effect of two different androgens and estrogens on the incidence of dental caries in gonadectomized and unoperated rats, and compare the effect on fluorine storage in the skeleton.

EXPERIMENTAL

Aproximately 300 weanling rats* were divided as to weight and sex into 3 experimental series. Each series consisted of 3 groups of each sex. In series 1, Group 1 were unoperated animals receiving testosterone† (2.5 mg. per week per animal of aqueous suspension), Group 2 consisted of unoperated animals receiving methylandrostenediol† (2.5 mg. per week per animal of aqueous suspension), and a third group served as controls. Series 2 was composed of one group of gonadectomized animals receiving the same doses of testosterone, another gonadectomized group receiving the same doses of methylandrostenediol, and a third group which served as controls. The third series consisted of an unoperated group which received diethylstilbestrol†

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*Sprague-Dawley Strain, furnished by Carl Wilson, Beech Grove, Ind.

†The authors wish to thank the Schering Corporation, Bloomfield, N. J., and Mattox and Moore, Indianapolis, Ind., for generously supplying the hormones used in these studies.

(1.5 mg. per week per animal in cottonseed oil), and a gonadectomized group receiving diethylstilbestrol at the same concentration, while a third group acted as a control. All animals were placed on the cariogenic diet[4] at 15 to 18 days of age. The groups that were gonadectomized were operated between 20 and 30 days of age. Diet (F = 0.5 ppm) and fluorine-low (F = 0.1 ppm) water were available to all the animals ad libitum. When the animals were 170 days old, they were sacrificed by chloroform inhalation, and the heads removed for caries evaluation and the femurs for fluorine analysis.

DATA AND DISCUSSION

The effect of the different androgens and estrogens used in this study on the incidence of dental caries in the rat is summarized in Table I.

TABLE I

EFFECT OF TESTOSTERONE, METHYLANDROSTENEDIOL, AND DIETHYLSTILBESTROL ON DENTAL CARIES INCIDENCE AND FLUORINE STORAGE IN THE UNOPERATED AND GONADECTOMIZED RAT

GROUP	NUMBER OF ANIMALS	FINAL WEIGHT (GM.)	NUMBER OF LESIONS	EXTENT OF LESIONS	SKELETAL ANALYSIS (ASHED FEMUR)		
					WEIGHT (GM.)	F CONC. (μG/GM.)	TOTAL F (MG.)
Males							
Series I							
Control	13	276	9.2	2.3	0.3518	163.8	0.057
Control + test	15	241	10.2	2.7	0.3491	113.5	0.039
Control + MAD	13	245	8.4	2.1	0.3144	93.4	0.025
Series II							
Control	18	361	8.4	2.0	0.3749	119.5	0.045
Castrate + test	19	311	8.8	2.1	0.3709	126.6	0.047
Castrate + MAD	17	301	8.1	2.0	0.3341	106.4	0.035
Series III							
Control	16	333	6.2	1.5	0.3368	104.8	0.036
Control + DES	15	172	9.1	2.4	0.2408	125.7	0.030
Castrate + DES	17	172	10.3	2.8	0.2275	148.7	0.035
Females							
Series I							
Control	18	191	7.8	1.9	0.2941	107.0	0.032
Control + test	18	186	8.4	2.0	0.3098	112.2	0.035
Control + MAD	20	216	7.8	1.9	0.3322	158.1	0.053
Series II							
Control	18	216	8.7	2.2	0.2787	131.0	0.036
Castrate + test	18	238	8.3	2.1	0.3205	122.4	0.040
Castrate + MAD	17	273	8.5	2.3	0.2830	108.5	0.031
Series III							
Control	14	208	7.1	1.9	0.2213	102.2	0.023
Control + DES	13	156	8.8	2.4	0.2085	134.0	0.028
Castrate + DES	12	150	10.2	2.8	0.2035	177.4	0.036

Testosterone appears to increase the caries experience in both unoperated males and females, while methylandrostenediol decreases caries experience in the males, but is without effect in the females. The effect of testosterone is similar to that reported previously.[1] However, analysis of the data indicates that the difference between the caries experience in the controls, and the controls receiving testosterone, is not significant in either the males or the

females. Similar statistical results were found for the males receiving methyl-androstenediol. When similar androgens were administered to gonadectomized animals no differences in the caries incidence were found. These facts, coupled with previous reports,[1] seem to indicate that the androgens are not effective in altering the normal caries experience in rats on a cariogenic diet beyond the normally experienced inherent variability.

The results of estrogenic hormones on dental caries in the rat are quite different. The administration of diethylstilbestrol to either control males or orchiectomized animals increases the caries experience. Except for one instance,[2] this corroborates previous work. A further extension of this fact is found in the data which show a similar result after the administration of estradiol in either control males or orchiectomized animals.[1, 2] Similar results are found in the females. Again, except in one instance,[2] the administration of either diethylstilbestrol or estradiol to either control female or ovariectomized animals results in an increased caries experience. The difference in caries experience, as seen in Table I, in those animals receiving the estrogenic hormones, are all significant at a 0.01 confidence level.* It appears, then, that the androgens are without effect on the dental caries experience in either males or females while the estrogens appear to increase the caries incidence significantly.

The administration of the respective hormones to gonadectomized animals does not modify these conclusions, but the caries increase appears even greater in these animals. A complete summarization of the pertinent existing data from these laboratories is seen in Table II. A wide variability exists between different control groups as to their caries experience. Not only are differences between groups noted, but differences among groups as to the sex of the animal also constitute a variable. In 50 per cent of the experiments, the males had a higher number of cavities than the females, although this difference was significant in only one instance, while in 50 per cent of the experiments, the females had higher numbers of cavities than the males. In no instance were these differences significant.

In all instances, orchiectomy reduces the incidence of dental caries, this difference being significant from the unoperated male control in each instance. In two experiments, ovariectomy increased the number of cavities, while in a third experiment, it reduced caries. In one experiment, where an increase in caries was reported, these were significantly different from unoperated female controls. Unoperated males given androgens increased the number of cavities in the males in two experiments and decreased the number in one when compared to control males, while in the females, there was an increase in one experiment and no difference in another. None of these differences in either the males or females are significant when compared to their respective unoperated controls. When androgens were administered to orchiectomized animals, a reduction in number of cavities was found in two experiments and

*As determined by the T test; see Snecedor, G. W.: Statistical Methods, Ames, Iowa, (1950), The Iowa State College Press, p. 75.

an increase in one, while a reduction in numbers of cavities was found in both experiments in which androgens were administered to ovariectomized animals when compared to unoperated controls. None of these differences in either the males or females are significantly different when compared to their respective unoperated controls.

When the estrogens were administered to unoperated males, there was an increase in the number of cavities in two experiments and a reduction in another. The increase in caries in both instances was significant, while the reduction was not. The effect of estrogens on unoperated females was to increase caries in three experiments and to reduce caries in one. The increase was significant in all instances, while the reduction in the one experiment was not. The effect of the estrogens in orchiectomized animals indicated

TABLE II

SUMMARY OF THE EFFECT OF DIFFERENT ANDROGENS AND ESTROGENS ON DENTAL CARIES INCIDENCE IN UNOPERATED AND GONADECTOMIZED RATS

GROUP I	DENTAL CARIES EXPERIENCE* (MEAN NUMBER LESIONS PER GROUP)					
			Males			
Control	8.0	6.9	8.7	9.2	8.4	6.2
Castrate	6.5	5.0	6.9	--	--	--
Control + test	8.9	--	--	10.2	--	--
Castrate + test	7.4	--	--	--	8.8	--
Control + DES	--	5.3	--	--	--	9.1
Control + estr.	--	9.8	--	--	--	--
Castrate + DES	--	--	9.6	--	--	10.3
Castrate + estr.	--	--	9.5	--	--	--
Control + MAD	--	--	--	8.4	--	--
Castrate + MAD	--	--	--	--	8.1	--
			Females			
Control	7.1	5.8	8.9	7.8	8.7	7.1
Castrate	8.4	7.0	8.1	--	--	--
Control + DES	6.5	8.1	--	--	--	8.8
Castrate + DES	9.0	--	9.9	--	--	10.2
Control + estr.	--	7.6	--	--	--	--
Castrate + estr.	--	--	9.4	--	--	--
Control + test	--	--	--	8.4	--	--
Control + MAD	--	--	--	7.8	--	--
Castrate + test	--	--	--	--	8.3	--
Castrate + MAD	--	--	--	--	8.5	--

*Only vertical columns can be compared to each other.

a significant caries increase in all three experiments, while the administration of similar amounts of estrogens to ovariectomized animals in four experiments similarly increased the caries experience in all cases. However, only two of four increases were significantly different from unoperated female controls. Thus, these data appear to indicate in a convincing manner that the effects of androgens and estrogens are different in their ability to affect the dental caries experience in rats. The mechanism by which this occurs is not presently understood, but judging from previous experiences in these laboratories, it would seem reasonable that the hormones may affect the salivary glands, or other endocrine organs which in turn may affect the salivary gland, with the result that the rate, volume, or consistency of salivary secretion is modified.

These studies seem of sufficient importance to warrant continued investigation of these problems, with the future purpose being to attempt to measure the effect of the endocrine system on salivary function.

The effect of the various androgens and estrogens on fluorine storage in the skeleton is seen in Table I. There appear to be no changes of sufficient magnitude to warrant serious consideration. The common finding that the concentration of fluorine is greater in the animals receiving the estrogens seems to be related to the over-all growth of the animal since these animals frequently have a smaller skeleton. The total fluorine, although significantly different in some instances, does not seem to be directly related to either the effect of dental caries incidence or a constant representation of one of the estrogens or androgens.

SUMMARY

Two androgens (testosterone and methylandrostenediol) and estrogens (estradiol and diethylstilbestrol) affect the dental caries incidence in immature rats in a different manner. In unoperated animals the androgens are without effect in altering the normally experienced caries rate, while the estrogens significantly increase the incidence of dental caries. When the animals are gonadectomized and then receive the different hormones, no difference is noticed by androgen administration, while the effect of the estrogens appears to be more pronounced.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Proceedings of the Thirty-Third General Meeting
Chicago, Illinois

March 18, 19, and 20, 1955

Compiled by Hamilton B. G. Robinson,* Editor, College of Dentistry, Ohio State University, Columbus, Ohio

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I. Introductory Statement

The 33rd General Meeting of the International Association for Dental Research was held on March 18, 19, and 20, 1955, preceding the annual meeting of the American Association of Dental Schools. One hundred and seventy-three papers were presented in the general meeting. The general meeting was divided into 15 "sections": anatomy and surgery, animal caries, composition of calcified tissues, cytology and histology, dental pulp, fluorides and dentifrices, growth and metabolism, histochemistry and enzymes, human caries, microbiology, neoplasms, periodontal disease, saliva, and miscellaneous. In addition, 37 papers were presented to the materials group and 54 papers were read by title.

On Saturday evening a dinner was held at which Dr. Daniel F. Lynch, President of the American Dental Association, spoke on the role of research in dentistry. The Novice Award was given to D. E. Waite and C. E. Staley of the University of Iowa. The first Wilmer Souder award was made to Richard L. Coleman. On Sunday afternoon the newly elected President, Paul E. Boyle of the University of Pennsylvania, narrated his trip to Burma. On Sunday evening Dr. Harold Urey addressed a joint session of the International Association for Dental Research and the American Association of Dental Schools.

II. Index of Participants and Sequence Numbers for Abstracts

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III. Abstracts of Papers Read in General Meeting Sessions

1. Chloride in Saliva. *John T. Anders, B.D.Sc., Biochemistry Department, Forsyth Dental Infirmary for Children, Boston, Mass.* A method for measuring the chloride-ion concentration of the saliva and a classication of subjects according to the DMF index with special regard to caries activity were presented. The measurement of the chloride-ion concentration is based on the titration of deproteinized saliva against mercuric nitrate using diphenylcarbazone as an indicator. Two hundred and fifty-one subjects from 3 years, 6 months to 15 years, 2 months old were tested. The range of salivary chlorides varied from 6.49 to 37.88 meq. Cl per liter. Subdividing the group tested according to caries activity, it was found that the 30 caries-free subjects had an average of 12.59 meq. Cl per liter while the 80 cases with extensive, active, rapid decay had an average of 18.31 meq. Cl per liter. The moderate groups fell in between these values. A high chloride level in the saliva is apparently associated with high caries incidence. The possible implications of the presence of the chloride ion in the saliva are discussed in the light of several in vitro studies which have indicated that chloride is an activator for amylase.

2. Transplantation of Teeth—Histological Studies. *R. Gordon Agnew, D.D.S., M.Sc.(Dent.), Ph.D., and Chester Fong, D.D.S., College of Dentistry, University of California, San Francisco, Calif.* Successful transplantation of the developing third molar into the socket of the lower first molar in the human being has been reported by one of us (C. F.). The present report is part of a current study covering microscopic interpretation at postoperative intervals up to 2 years; it deals with findings at 4-day, 4-week, and 4-month periods following transplantation, in the rhesus monkey, of third molars which had reached the stage of beginning root formation. Four-day findings: the third molar lies in the enlarged socket, the roots lightly contacting bone at several points. Organizing clot fills the interradicular space, with vigorous fibroblast proliferation. Active granulation tissue directly contacts wide pulpal openings and envelops lower portion of outer root aspects. Cervically, circumdental area as yet shows no organization. The pulp shows no mass tissue necrosis. Changes are least near the wide apical openings. In the pulp chamber there is some edema, paucity of cells, and areas of fine fibrillar tissue. Four-week findings: the tooth, not yet in occlusal contact, is substantially advanced in normal integration. The developing periodontal membrane and the gingivae are approaching functional status. In the canals the pulp appears healthy. Areas remote from the apices are rather coarsely fibrillar. A narrow band of "osteodentin" lines the dentin walls along which there are numerous odontoblasts. At the root tips odontoblasts lie against earlier irregular deposits of osteodentin. The processes are in harmony with clinical evidences of successful transplantation. Four-month findings: massive osteodentin formation has narrowed the pulp chamber and 1 canal. Another canal shows, within the pulp tissue, a long bony "spike" continuous with the periapical bone. Hypercementosis has occurred. The periodontal tissues are intact. In experimental transplantation the establishment of periodontal integrity appears to be readily achieved. However, the status of the pulp seems highly significant in determining the extent and character of root growth, dentin formation, and the integrity of the pulp cavity.

3. Histopathology of Dental Caries in Human. *Sumter S. Arnim, D.D.S., Ph.D., Dental Branch, University of Texas, Houston, Tex.* Studies of partly demineralized serial sections of carious human teeth provide additional data concerning the mechanism of the carious process. The technic of preparation and sectioning preserves the bacterial plaque, cavity content, enamel remains, and the other tissues in their natural relations. The plaque is composed primarily of bacteria. Recognizable remnants of food débris are seldom seen. Mucin stains reveal no mucin content. Changes occur in enamel ahead of bacterial invasion. The lesions are characterized by: (1) presence of bacterial plaque; (2) loss of translucency; (3) cavitation, micro- and macroscopic; (4) color changes related to staining technic and influx of foreign substances from the mouth into the microscopic porosities; and (5) gross cavitation. There is a typical spread of the lesion at the dentinoenamel junction with invasion of dentin. Dentinal changes are seen in advance of bacterial invasion. No specific relation is observed between organic elements and demineralization of enamel or dentin. Lamellae appear to be permeable to demineralizing agents but no evidence is found indicating a significant relation to spread of the carious lesion. The carious process demineralizes enamel regardless of lamellae. Cracks in enamel filled with particles resembling bacteria are seen frequently without evidence of carious changes.

4. Radioiodine in Traumatized Rat Molars. *M. M. Ash, D.D.S., M.S., School of Dentistry, University of Michigan, Ann Arbor, Mich.* The relative

rate of uptake of radioiodine by traumatized rat molars was investigated using an intraperitoneal injection of I^{131}. A molar on one side of the lower arch was traumatized with excessive action of a gold-foil plugger; a molar on the other side of the arch was used as a control tooth. Traumatization was started 10 days prior to injection of the radioactive tracer. The relative activity of I^{131} was measured in the molars, thyroid gland, parotid gland, kidney, liver, and other tissues after 6, 12, 24, 36, and 48 hours from time of injection of the radioiodine. All activity measurements were corrected for decay. The peak radioiodine uptake was reached by the traumatized molars before any of the other tissues. The uptake of I^{131} by the traumatized molars was 3 to 5 times as great as for the untraumatized molars.

5. Acid Mucopolysaccharides and Nucleic Acids in Developing Teeth. *James K. Avery, D.D.S., Ph.D., School of Dentistry, University of Michigan, Ann Arbor, Mich.* A study of the location of acid mucopolysaccharides and nucleic acids as correlated to the various stages of tooth development was carried out on the teeth of the domestic pig. A series of tissue sections of developing teeth ranging from early cap stage to eruption were stained in 0.5 per cent solution of toluidine blue buffered at pH 4.0, 5.0, 6.0, and 7.0. No metachromasia was exhibited in the developing organs or surrounding connective tissue at any pH used until the bell stage of development. During dentino- and amelogenesis, increasing amounts of acid polysaccharides were evident in the ground substance of the surrounding follicle, stellate reticulum, dentinal tubule, and pulp area. The tooth-formative cells and enamel appeared uniformly orthochromatic. Alternate tissue sections, before staining in toluidine blue, were incubated in ribonuclease. A resultant lack of orthochromatic staining of the odontoblasts and ameloblasts in these tissues indicates a high concentration of nucleic acids in the cytoplasm of these cells.

6. Comparative Observations on Certain Histochemical Characteristics of Keratinous Tissues of Fish, Reptiles, Birds, and Mammals. *Russell J. Barrnett, M.D., and Reidar F. Sognnaes, D.M.D., Ph.D. (Technical assistance by George Pettengill), Department of Anatomy, Harvard Medical School, and the Harvard School of Dental Medicine, Boston, Mass.* Specific histochemical methods for sulfhydryl and disulfide groups have been applied to a study of epidermis and epidermal derivatives of fish, reptiles, birds, and mammals to ascertain the distribution of these groups in prekeratinous and keratinous tissues and to determine whether or not a pattern of these reactions existed that would correspond to the division of keratins into "hard" and "soft" varieties. The conclusion is reached that this conventional division is not generally applicable as it concerns sulfhydryl- or disulfide-containing protein for the following reasons. It was found that both sulfhydryls and disulfides are present at the prekeratinous stage of cornification; that all keratinized tissues, except some animal hairs, contain free sulfhydryls; and that only some of these groups are oxidized to disulfide bonds during keratinization; that a "keratogenous zone," rich in sulfhydryl, and supposedly only occurring in "hard" keratins, is absent in some "hard" and present in some "soft" keratins; that, although "soft" keratin (epidermis) has a transitional zone histologically between the Malpighian and keratinized layers, it contains no such zone histochemically; that keratohyaline and trichohyaline granules contain no sulfhydryl or disulfides; and that the distribution of these chemical groups in hair cortex, frequently a model for "hard" keratin, is quite different than that in other hard keratinous structures such as claws,

horns, hoofs, feathers, and scales. Since "hard" and "soft" have frequently been used to imply more than texture of keratins and since these proteins are heterogenous chemically, it may be advisable to classify them by the specific anatomic structure and animal in which they occur.

7. Effects of Tryptophan Deficiency on the Bones and Teeth of Rats. *L. A. Bavetta, Ph.D., and S. Bernick, M.S., School of Dentistry and School of Medicine, University of Southern California, Los Angeles, Calif.* Weanling rats were placed on a tryptophan-deficient diet for periods varying from 4 to 26 weeks. The effect of the deficiency upon the epiphyseal area of the long bones, alveolar bone, and dentin was studied. A progressive atrophy of the epiphyseal plate was noted after 4 weeks of tryptophan deficiency. Prolongation of the deficiency to 8 weeks produced a calcification of the matrix of the epiphyseal cartilage to the depth of a few cells on the diaphyseal side. This "bony seal" was extended to a thin plate of bone across the epiphyseal disc in animals that were tryptophan-deficient for periods of 16 to 26 weeks. Osteoporosis of long bones as well as alveolar bone also occurred. In addition to these bony changes there was a marked hypocalcification of the dentin. The osteoporotic effects were proportional to the duration of the deficiency. However, what appears to be a compensatory calcification of alveolar bone occurred in some of the animals longest on the deficiency. This may be a response of a very much weakened bone to the traumatic stress of occlusion. A second group of rats was placed on a tryptophan-deficient diet at ages varying from 7 to 26 weeks. The severity of the reaction on both bones and teeth was much less in the older animals.

8. Histochemical and Electron Microscopy Studies on Tryptophan-Deficient Dentin. *Sol Bernick, M.S., Lucien Bavetta, Ph.D., and Richard Baker, School of Medicine and School of Dentistry, University of Southern California, Los Angeles, Calif.* In a previous study (Bavetta and Bernick, 1954) the authors demonstrated that in rats fed a tryptophan-deficient diet the dentin showed an increased number of interglobular areas, characteristic of poor calcification. In the present study histologic sections from both the control and tryptophan-deficient animals were treated by the following histochemical technics: (1) periodic Schiff reaction; (2) toluidine blue for metachromasia; (3) alkaline phosphatase; (4) coupled tetrazonium reaction. The results of the periodic Schiff reaction and toluidine blue staining indicate the depolymerization of the ground substance of the dentin which prevents proper calcification. The findings of the coupled tetrazonium reaction reveal the presence of tryptophan as a component of the ground substance, and its lack is probably a factor in the depolymerization of the ground substance. When comparing the alkaline phosphatase staining in the control and deficient animals, it was noted that there is a decrease in enzymatic activity in the deficient animals. Electron microscopy studies reveal the presence of collagen fibrils of 650 A striations which in the normal dentin is masked by the unaltered ground substance.

9. Morphology and Histochemistry of Osteoclasts. *S. N. Bhaskar, D.D.S., Ph.D., C. Mohammed, L.D.S., M.S., J. P. Weinmann, M.D., and I. Schour, Ph.D., D.D.S., College of Dentistry, University of Illinois, Chicago, Ill.* Serial histologic tracings and histochemical study of osteoclasts from the region of the sockets of developing teeth were made in the mice, rats, and *ia* rats. The findings and conclusions were: (1) In areas of rapid bone resorption, osteoclasts tend to form a syncytium. (2) Osteoclasts may possess long

processes which may or may not contain nuclei. Large portions of these cells are nucleus-free. In histologic sections, therefore, which pass through the nucleus-free portions, the osteoclasts are not recognized. If the sections pass through a nucleated process, these cells may be mistaken for osteocytes or osteoblasts lying in areas of resorption. (3) Since osteoclasts are observed in areas in which bone resorption does not occur (*ia* strain), it can be assumed that osteoclasts do not originate by the fusion of liberated osteocytes and do not necessarily appear subsequent to bone resorption. 4. Thionin staining showed that the cytoplasm of the osteoclasts in the *ia* strain of rats contained less RNA than the osteoclasts of normal rats, thus suggesting a difference in metabolic activity. (5) Feulgen staining indicated a greater incidence of irregularity in the outline of the nuclear membrane of the osteoclasts in the *ia* strain of rats.

10. Tumors of the Minor Salivary Glands. *S. N. Bhaskar, D.D.S., Ph.D., and J. P. Weinmann, M.D., College of Dentistry, University of Illinois, Chicago, Ill.* Whereas numerous cases and classifications of major salivary gland tumors have been reported, tumors originating in the minor glands of the oral cavity have not been studied systematically. The present report on the morphology of tumors involving the salivary glands of the lip, cheek, palate, alveolar mucosa, and the fornix of the vestibule is based on 23 cases. To demonstrate the cellular components of the tumors, sections were stained with hematoxylin-eosin, Mayer's mucicarmine (counterstained with hematoxylin), erythrosin-safranine, and van Gieson's method. Of the 23 tumors, 4 were pleomorphic adenomas, 4 canalicular adenomas, 3 myoepitheliomas, 1 mucocyst adenoma, 3 fibroadenomas, 6 mucoepidermoid tumors, and 2 adenocarcinomas. The relatively rare canalicular adenomas and myoepitheliomas consist exclusively of ductal cells and myoepithelium, respectively. It is demonstrated again that the pseudocartilage of the pleomorphic adenoma is a mixture of mucin and stromal tissue. In the present study the most common site of the tumors of the minor salivary glands is the palate and the most common neoplasm, the mucoepidermoid tumor.

11. Changes in the Nitrogen Content of Enamel With Age. *B. R. Bhussry, D.M.D., and V. Emmel, Ph.D., School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* This study attempts to correlate an increase in the amount and intensity of pigmentation with increasing age to the possible variations in the nitrogen content of sound enamel in teeth of different ages. Extracted teeth were divided into 6 groups with 5 years' difference in their posteruptive ages. All the available history was recorded, ground sections prepared, and enamel separated from these by using a thin jet of abrasive from the "Airdent" machine. Enamel samples weighing 150 to 200 mg. were used from each tooth for Kjeldhal determinations of nitrogen. Enamel from teeth showing surface changes other than caries were also used. Analysis of the data shows a definite relative increase in the nitrogen content of enamel with increasing age as determined on a weight basis. The same is true of the brown-stained enamel in the proximal areas of the teeth. This increase in the nitrogen content of enamel, and the corresponding increase in the amount and the intensity of pigmentation in sound enamel with increasing age suggests an organic change in enamel. This might explain not only the variations in color of younger and older teeth but also the greater resistance of older pigmented enamel to decalcification with acids.

12. The Histochemistry of the Adrenal Cortex Following Desalivation. *David Bixter, B.S., Joseph C. Muhler, B.S., D.D.S., Ph.D., and Richard*

C. Webster, Indiana University, Bloomington, Ind. Experiments in this laboratory have shown that desalivation was accompanied by uterine and testis weight alterations as well as by histologic changes in the adrenal gland. These findings, in addition to those of other workers, indicate that the salivary glands may have an intimate relationship to the various endocrine glands. To extend these results the adrenal glands of desalivated, sham-operated, and control rats were studied histochemically at various ages. The uteri and ovaries were also examined histologically for any changes accompanying desalivation. The results obtained with the sudan stains indicated an increased lipoid activity in the cortex of the desalivated animals during the first 6 days following the operation. Similarly, the Schiff plasmal reaction was most intense in the fasciculata of the desalivated animals and a pronounced increase in fine birefringence was also observed in this zone during the same period. The control and sham-operated animals failed to indicate any of these alterations. From the 10th to the 32nd experimental day no histochemical differences were evident between any of the groups. The uterus of the desalivated animal was considerably smaller and less well developed than that of the other groups. Removal of the salivary glands was also accompanied by a reduction in ovarian size and a marked reduction in the number of corpora lutea. Removal of the major salivary glands is accompanied by an increased adrenocortical activity as evidenced by histochemical tests. In addition, the development of the ovary and uterus is retarded by desalivation.

13. The Effect of Sucrose Upon the Solubility of Human Dental Enamel. *R. Q. Blackwell, Ph.D., and L. S. Fosdick, Ph.D., Northwestern University Dental School, Chicago, Ill.* The possibility generally has been ignored that sucrose may affect the caries process in other ways in addition to its accepted role as substrate for acid formation. In the present in vitro experiment the effect of sucrose upon enamel solubility was studied. Powdered human enamel was added to lactic acid buffers of graded pH values in the presence of, and in the absence of, graded amounts of sucrose. The suspension of enamel in solution was agitated at constant temperature for varying intervals of time. The resulting solutions were analyzed for content of calcium ion (and in some instances phosphate ion). The calcium (and phosphate) content of the solution was taken as an index of the amount of enamel dissolved. The results clearly indicate that, in vitro, the presence of sucrose in the lactate buffer solutions lowers the rate of dissolution of powdered human dental enamel. For example, at pH 4.5, the amount of calcium in solution after 5 minutes in the lactate buffer (32.0 mg. Ca^{++} per 100 ml. of buffer solution) is approximately equal to the amount found after 10 minutes in the pH 4.5 lactate buffer containing 0.5 molar sucrose (33.4); the amount dissolved after 30 minutes by the pH 4.5 lactate buffer containing 1.0 molar sucrose was only slightly greater (36.4). Possible explanations for the results noted may be considered under the headings of viscosity effects, coating effect, complexing or chelating action, hydration effects. While all these effects may be operating, it is difficult to assess their relative magnitude. The present work suggests that caries development may be influenced by the presence of sucrose in dental plaque in 2 opposing ways: (1) the sucrose acting as a substrate for acid formation to promote decalcification, and (2) the sucrose acting to diminish the rate of enamel decalcification by the acid. The relative importance of the first effect would be expected to be much greater than that of the second.

14. Analeptic Efficacy in Short-Term Barbiturate Anesthesia. Walter *Bogdanski, D.D.S., School of Dentistry, Georgetown University, Washington,*

D. C. Increased use of ultrashortacting barbiturates in office anesthetic procedures has emphasized the indication for curtailment of postanesthetic barbiturate depression. The efficacy of analeptic drugs in this regard was investigated. Seventy-five patients were divided into three groups which were similar in that individuals of the same sex and near-equal age appeared in each group. Like amounts of Pentothal sodium were administered intravenously to persons of similar age and size in each group. On return of the corneal reflex 1 group received intravenously 300 mg. of caffeine and sodium benzoate, another 10 mg. of dextroamphetamine sulfate, while the 3rd acted as a control. Time elapsing from return of corneal reflex until the following neuromuscular skills could be performed was measured: (1) touch the nose 6 times with the alternate index fingers, (2) make 8 successive heel to toe steps along a straight line, (3) follow the course of a pencil passed before the eyes without evidence of nystagmus. The mean value of these times was computed and the groups compared.

15. Radioisotopes in the Qualitative Study of Dentinal Sclerosis. *Julian D. Boyd, M.D., Veterans Administration Hospital, Coral Gables, Fla.* Sections of teeth were immersed in solutions of radioiodine and of radiophosphorus; then autoradiograms were made. These records were compared with the physical characteristics of the tooth substance as revealed through macrophotographs of the same section, with and without staining with basic fuchsin. Comparison was made between sound teeth and teeth with filled or unfilled areas of caries. The findings indicate that the radioisotope is taken up in a manner similar to stain: that in regions of dentinal sclerosis the uptake of each is less than in areas of nonsclerosis. The methods complement each other in revealing the apparent rate at which caries is progressing, in specimens showing rapid destruction, and in others showing definite arrest of caries with sealing of the subjacent dentinal tubules, hyalinization of the dentin, and the deposition of secondary dentin in the pulp chamber.

16. Decreased Vascularity of the Dental Pulp: Its Effect on the Dental Caries Experience in Rats. *Harold E. Brewer, B.S., Joseph C. Muhler, D.D.S., Ph.D., and Robert B. Fischer, Ph.D., Indiana University, Bloomington, Ind.* Ligation of arteries supplying the maxillary and mandibular molars of weanling rats results in both (1) a significant increase in the number of carious teeth and (2) an increase in the extent of the carious lesion as compared with identical unoperated controls. The operation consisted of bilateral ligation of external and internal maxillary arteries. Operation success was verified by injecting a liquid latex solution central to the site of ligation and by following the injection mass to the ligation point macroscopically. Histologic support is further obtained by comparison of the pulpal tissues in both groups. A cariogenic diet (F = 0.5 ppm) and tap distilled water (F = 0.05 ppm) were available to both groups ad libitum. Dental caries experience was determined by visual observation, using a binocular microscope, according to methods previously reported.

17. Distribution of Lead and Tin in Human Enamel. *Finn Brudevold, D.D.S., M.S., and Ludville T. Steadman, Ph.D., Eastman Dental Dispensary and University of Rochester, Rochester, N. Y.* Successive layers of enamel were ground from unerupted and erupted teeth of different ages and subjected to spectrographic analysis of lead and tin. Lead was present in the enamel of all groups of teeth and occurred in greatest concentrations in the outer enamel and

decreased in concentration in subsurface layers to a constant level of 30 to 90 ppm in the bulk of the enamel. The lead concentration in the outermost enamel of fully formed unerupted teeth was greater than that found in incompletely formed ones, and a further increase appeared in the outer enamel of erupted teeth with age, the greatest concentration found being 550 ppm in teeth over 50 years of age. Tin was found in concentrations of 1 ppm and was evenly distributed in the enamel of unerupted teeth. Only 7 ppm was found in the external enamel of persons over 50 years of age. A greater tin content was observed in enamel from teeth filled with amalgam than in enamel from intact teeth. Tin is readily acquired by enamel exposed to solutions of stannous fluoride (pH 2.9) and stannous chlorofluoride (pH 1.6). It is believed that a low pH is necessary for tin uptake.

18. The Reducing Activity of Hetero- and Homofermentative Lactobacilli. *George W. Burnett, M.A., D.D.S., Ph.D., and Eleanor G. Schmidt, M.S., Department of Dental Research, Army Medical Service Graduate School, Washington, D. C.* The reducing activity of 5 strains of heterofermentative and 10 strains of homofermentative lactobacilli was investigated by the use of 2, 3, 5-triphenyltetrazolium chloride (TTC), which upon reduction changes from a colorless form to the red, water-insoluble compound, formazan. TTC was optimally reduced by most strains of both metabolic types of lactobacilli from an 18-hour culture in 0.2 M phosphate buffer at pH 6.0, utilizing 0.1 per cent TTC, M/50 glucose, and at 45° C. While the rate of reduction of TTC varied slightly with the lactobacillus strain, optimal conditions for TTC reduction were essentially the same for both homo- and heterofermentative types. The similarity of optimal conditions indicates that the same factors in each metabolic type are probably concerned in the reduction of TTC but that the amount of such factors varies consistently between each of the types. The minimum cell concentration, as determined by nitrogen content, which discernably reduces TTC, is consistently less for the heterofermentative strains than it is for homofermentative strains. The rate of TTC reduction increases rapidly as the cell concentration of heterofermentative lactobacilli increases in the test solution, in contrast with the slow increase in reduction rate when the cell concentration of the homofermentative lactobacilli is increased. The differential rates of TTC reduction can be utilized to differentiate homo- from heterofermentative strains of lactobacilli.

19. A New Histochemical Method for Demonstration of Protein Groups. *M. S. Burstone, D.D.S., M.A., National Institute of Dental Research, National Institutes of Health, Public Health Service, U. S. Department of Health, Education and Welfare, Bethesda, Md.* 2, 4-dinitrofluorobenzene (DNFB) combines quantitatively with primary amino groups of proteins, the phenolic OH group of tyrosine, and the imidazole group of histidine. Danielli first proposed the use of DNFB as a histochemical reagent, but his procedure does not appear to be reproducible. The present method which appears to be quite specific and yields good color intensity with tissue sections may be outlined as follows: Sections are treated with an alkaline 1 per cent DNFB solution in 90 per cent ethanol for 20 to 24 hours at room temperature and run through 4 changes of 90 per cent ethanol. The sections are reduced with 5 per cent aqueous sodium hydrosulfite, diazotized with nitrous acid, and coupled with 1-amino-8-naphthol-3, 6-disulfonic acid at an alkaline pH. Representative tissue sections as well as Coujard "model slides" were subjected to various blocking procedures (deamination, acetylation, and benzoylation) which were interposed prior to staining. Tissue components stain varying in-

tensities of reddish purple, with collagen, elastic tissue, and red cells exhibiting the greatest reaction. Acetylation and benzoylation completely abolish the reaction in tissue sections, and also in components of the model slides which contain known reactive groups. Deamination only partly diminishes the reaction in tissue sections, presumably because of the remaining reactivity of tyrosine- and histidine-containing structures. This histochemical procedure is applicable to the visualization of protein group changes in normal and abnormal hard and soft tissues.

20. A Chromometer for Oral Tissues. *P. Burwasser, D.D.S., Ph.D., and T. J. Hill, D.D.S., School of Dentistry and Institute of Pathology, Western Reserve University, Cleveland, Ohio.* The recognition of variations in the color of normal, ischemic, and inflamed oral tissues, especially in the gingivae, has long been a subjective procedure. An objective method for recording color variations would be helpful in diagnosis, treatment, and experimental work. Matching of gingival color with test swatches is helpful, but is limited by the number of color samples available. The chromometer makes use of polaroid color filters. These, in combination with each other or with neutral filters, give an infinite series of shades in the red, red-blue, and red-blue-yellow ranges. It is thus possible to record, duplicate, and compare the exact color of any oral tissue. A series of observations is now being conducted for calibration purposes and to determine the range of the instrument. We feel certain that the chromometer will be of value in the fields of dermatology and ophthalmology as well as in epidemiologic studies of oral tissues.

21. Enamel Formation in the Rat Incisor. *Earl O. Butcher, M.A., Ph.D., and A. Cecil Taylor, M.A., Ph.D., College of Dentistry and the Graduate School of Arts and Science, New York University, New York, N. Y.* Enamel formation has been investigated in the rat incisor. In cross sections of the tooth the ameloblasts, aligned in a row, are seen to give rise to enamel rods whose axes are inclined 45 degrees from the axes of the ameloblasts. The succeeding row of ameloblasts produce enamel rods which are inclined in the opposite direction to those rods produced by the previous row. Thus alternate rows of rods are nearly at right angles to each other. In addition, all rods are bent about 30 degrees toward the apical end of the tooth. As a result of this formation, sagittal sections of the tooth frequently show rods cut nearly longitudinally alternating with rods cut transversely. The transverse sections of rods have been mistaken for globules by some investigators. They have also been interpreted as artifacts and in some instances false conceptions of enamel formation have been based on these cross sections. The ameloblast produces the cylindrical Tomes' process which has a thin wall and a homogenously staining center. The portion of each rod which is continuous with Tomes' process has similarly thin walls. The walls, however, rapidly become thicker with increasing distance from the ameloblasts until each rod becomes a solid structure. The homogenous portion of each Tomes' process thus assumes a conical shape with its apex extending into the core of the enamel rod.

22. The Retention of Various Foodstuffs After Eating. *Robert C. Caldwell, H.D.D., L.D.S., R.F.P.S., Thomas G. Ludwig, B.D.S., M.S., and Basil G. Bibby, B.D.S., Ph.D., D.M.D., Eastman Dental Dispensary, Rochester, N. Y.* The distribution of food retention on the mucous membranes and teeth was tested in 2 ways. In the 1st, acrylic splints were made to cover the teeth. After eating in the usual manner, the splints were inserted, the tongue and mucous membranes washed with water from the atomizer, the washings col-

lected for analysis. After removal of the splint, the teeth were similarly washed and the washings pooled with those from the inside of the splint. The carbohydrate content of the washings was determined by the anthrone method and food retention calculated. In a single subject the food retention on the mucous membranes and teeth was as follows: shortbread cookie, 385 mg., 230 mg.; date, 322 mg., 258 mg.; milk chocolate, 320 mg., 300 mg.; ice cream, 300 mg., 100 mg.; apple, 250 mg., 12.5 mg.; white bread, 125 mg., 80 mg.; cola beverage, 200 mg., 45 mg. The second method depended upon incorporation of a fluorescent substance in the foods and examination with ultraviolet light after eating. Various substances were tested, including b, methyl umbelliferone, ethyl di-ethyl amino coumarin, chlorophyll, and riboflavin. Riboflavin was chosen as the most suitable. It appears that liquids are more likely to lodge on the anterior teeth labially and to clear very rapidly. Semisolid foods were widely distributed and cleared slowly. Solid foods were retained more on the occlusal surfaces of the posteriors and cleared slowly.

23. Some New Halogen-Substituted Epinephrine Derivatives. *John A. Carbon, B.S., L. S. Fosdick, Ph.D., and R. Q. Blackwell, Ph.D., Northwestern University Dental School, Chicago, Ill.* Even though epinephrine has long been used with great success in conjunction with local anesthetics, workers have sought other compounds which would possess high vasoconstrictor properties but which would be free from the undesirable side effects of epinephrine. Many substitutes have been introduced onto the aromatic nucleus of epinephrine in order to achieve this goal, but the introduction of halogen atoms has received comparatively little attention. This work is an extension of the early work of Hansen, and later of Fosdick, Fancher, and Urbach, on the preparation of halogen-substituted epinephrine derivatives. We have prepared 2-chloro-4-hydroxy-α-(methylaminomethyl) benzyl alcohol hydrochloride and its corresponding ethylamino derivative, and have compared their pharmacologic properties to those of epinephrine. The chlorine atom placed in the 2-position seems to almost completely hinder vasopressor activity and, at the same time, greatly reduces the toxicity when injected intravenously.

24. Phosphatase Activity in the Salivas of Caries-Free and Caries-Active Individuals. *W. J. Carter, D.D.S., M.D.S., H. R. Englander, D.D.S., M.P.H., K. C. Hoerman, D.D.S., M.D.S., I. L. Shklair, Ph.D., and L. Stein, B.S., University of Kansas City School of Dentistry, Kansas City, Mo., and the Dental Research Facility, Great Lakes, Ill.* Fitzgerald suggested (1952) that, since the phosphatase enzymes in saliva were, for the most part, of bacterial-cell origin, an increase in concentration of these ferments might be an expression of certain oral debilities. Upon this premise the present experiment was conducted. Twenty-five milliliters of paraffin-stimulated saliva samples were collected from 100 male naval personnel whose average age was 19 years. Individuals with acute oral infections were excluded from the group. Similar samples were collected from 26 caries-free and caries-active recruits reporting for duty. Phosphatase activity tests were accomplished using the technique of Seligman and Chauncey. Quantitation was carried out employing the Coleman Junior Spectrophotometer. The wave length was 525 mμ; cuvette 10 by 75 mm. The means of acid and alkaline phosphatase activity in the salivas of 100 individuals were 0.57 and 4.72 units per 100 ml. of saliva, respectively. The differences in acid and alkaline phosphatase activity in caries-free and caries-active salivas were treated statistically and were found not to be significant. It was concluded that tests for these enzymes in saliva are not a satisfactory index to dental caries activity.

25. Experimental Lathyrism in the Rat: An Investigation of the Effect on Some Oral Structures. *Glenn R. Carwell, D.D.S., College of Dentistry, State University of Iowa, Iowa City, Iowa.* Lathyrism is a disease that results from the consumption of certain legumes of more than a dozen members of the genus Lathyrus. Symptoms of lathyrism include lameness, spinal curvature, sternal curvature, enlargement of the costochondral junctions, malformation and abnormal red color of the long bones, hernias, and dissecting aneurysms of the aorta. Every disorder observed in rats fed with *Lathyrus odoratus* seeds has a counterpart in diseases in humans. Many efforts have been made to isolate the toxic factor present in the Lathyrus pea. Schilling and Strong isolated a crystalline factor identified as B-(y-L-glutamyl) aminopropionitrile. The toxic factor apparently is the aminopropionitrile portion of the molecule. Reported oral findings in lathyrism are limited to (1) notching of the incisors and (2) exostoses of the lateral border of the mandible. Since definite lesions of mesodermal tissues of lathyritic rats have been observed and reported, it would be of some interest to learn if this syndrome affects the tissues of like embryonic origin present in the oral and dental apparatus. A study is now being made of the oral tissues in lathyritic rats to determine what effect lathyrism has on oral tissues.

26. Parotid Gland Secretions: Flow Rate and Interrelationship of Salivary Components. *Howard H. Chauncey, M.A., and Vincent F. Lisanti, D.M.D., Tufts College Dental School, Boston, Mass.* Stimulated parotid saliva was collected from healthy individuals using flavored gum base as the activation agent. A total of 4 samples were obtained, 2 samples each being procured on successive days. A significant increase in rate of flow was observed between the first sample and the remaining 3 samples. Once familiarization with the parotid cap was established, no significant difference in flow rate between ensuing samples occurred. Parotid saliva flow rates ranged from 0.30 ml. to 1.78 ml. per minute, with a mean rate of 0.72 ml. per minute. Analysis of the data showed a significant positive correlation between flow rate and pH, lactic acid, bicarbonate, sodium, calcium, and total proteins. A negative correlation was exhibited between parotid flow rate and potassium content. Interrelationships were noted between the various salivary constituents.

27. Relation of Structure to Cholinergic Action in a Series of Uncharged Carbon Compounds. *John P. H. Clark, M.S., D.D.S., School of Dentistry, Western Reserve University, Cleveland, Ohio.* The carbon analogue of acetylcholine, tertiary butylethyl acetate, which is a neutral compound incapable of ionization, was tested to determine whether molecular form alone is capable of conferring an appreciable degree of cholinergic activity. As determined by the increase in tonus of segments of the small intestine of rabbits, tertiary butylethyl acetate was 0.32 per cent as active as acetylcholine, isoamyl 0.11 per cent, and normal amyl 0.02 per cent; all being reasonably predictable. Normal propyl acetate was 0.14 per cent as active but unpredictable. Compounds, bearing less structural resemblance to the acetylcholine cation exhibited less activity and were unpredictable. Sufficient atropine to inhibit the action of acetylcholine also inhibited the action of the acetates. Tertiary butylethyl acetate caused a decrease in rate and amplitude of contraction of the frog heart, being about 0.25 per cent as active as acetylcholine. Isoamyl and normal amyl acetate were also active. This action was also blocked by atropine. None of the compounds caused a fall in the blood pressure of a cat when injected intravenously nor a contraction of eserinized rectus abdominus muscle of a frog. Tertiary butylethyl acetate, like acetylcholine, caused a

contraction of strips of uterus taken from nonpregnant guinea pigs. The activity was about that shown on the rabbit intestine and frog heart. Teritary butylethyl acetate was hydrolyzed almost as rapidly as acetylcholine by so-called "true" or "specific" cholinesterase obtained from human red blood cells.

28. Ammonia Production in Saliva. *C. T. Clegg, Phm.B., and J. J. Rae, Ph.D., Department of Chemistry, University of Toronto, Toronto, Canada.* A. Saliva-glucose experiments. Previous work has shown that saliva on incubation produces ammonia. This ammonia production can be inhibited by the addition of glucose in concentrations as low as 0.1 per cent. If the saliva is buffered at pH 7.0, this glucose inhibition is still observed, showing that this interference with ammonia production is not entirely due to a lowering of the pH. B. Glucose urea-saliva experiments. There is in saliva an active urease which produces large amounts of ammonia when urea alone is added to saliva in low concentrations (0.1 per cent). High concentrations of urea (10 per cent) inhibit ammonia production. If glucose is added to these urea-saliva mixtures the ammonia produced is greater than that obtained when urea alone is used. If buffered solutions are used in these experiments (pH 7.0) the stimulating effect of the glucose on the ammonia production is not observed, indicating that this stimulation could be attributed to a pH effect.

29. The Gingiva at Puberty. *M. Michael Cohen, D.M.D., Department of Pediatrics, Tufts College Medical School; Department of Oral Pediatrics, Tufts College Dental School; and The Boston Floating Hospital, Boston, Mass.* Two hundred and seventy boys and girls, aged 11 to 14, at St. Mary's School in Brookline and Myles Standish Camp in Taunton, Massachusetts, were examined clinically. Each case was charted carefully and the number of gingival papillae that were enlarged and inflamed noted. Oral hygiene, the occurrence of stain, calculus, and the occlusion of teeth were observed. In addition, the menarche and the appearance of pubic hair were recorded. From these observations, it became apparent that gingival enlargement in the anterior segment of the maxilla occurred with marked regularity in the prepubertal and premenarchial period, as well as in pubescence. The labial gingiva was most often involved while the lingual gingiva remained unaltered. The gingival enlargement was marginal in distribution and in the presence of local irritation was characterized by prominent bulbous interproximal papilla and was far in excess in size to gingival enlargements associated with local factor seen in development enlargement (ages 8 to 10) and gingival enlargement in the postadolescent period. Gingival enlargement in the anterior segment of the maxilla and mandible is an additional sign, together with secondary sex characteristics, of the beginning of pubescence. The role which the hormones of the anterior pituitary, adrenals, testes, ovaries, and local factors play in gingival enlargement in the prepubertal period and in pubescence is uncertain.

30. Degenerative Changes in the Articular Disc in Later Maturity. *Russell D. Coleman, D.D.S., and H. Henry Weisengreen, D.D.S., College of Dentistry, University of California Medical Center, San Francisco, Calif.* Ninety temporomandibular joints from 45 cadavers (36 men, 9 women) ranging in age from 39 to 95 years comprised the material for this study. Cause of death for each cadaver was recorded from hospital history. The presence of natural teeth and prosthetic appliances was compiled for each specimen. The bodies were decapitated, and the heads sagittalized and roentgenographed. Subse-

quently each joint was opened by incising around the circumference of the capsule between the disc and glenoid fossa. Care was exercised not to injure the disc. The condyle and disc were removed in toto. The discs were arranged into 2 groups according to the degree of macroscopic degenerative changes observed. Alteration in the articular disc was classified average degenerative change; the latter consisted either of smooth worn facets, or granular appearance of the upper surface of the disc. Discs exhibiting fraying, splitting, and perforation accompanied by eburnation of the condylar head were labeled maximal degenerative changes. Representative discs for each group were sectioned and stained with hematoxylin and eosin for purposes of tissue identification. From the 45 cadavers studied, 10 (22.2 per cent) exhibited either 1 or both articular discs with maximal degenerative changes. The latter changes were observed in 4 (44.4 per cent) female and in 6 (16.6 per cent) male cadavers whose average ages were 75.7 and 64.2 years, respectively. The lateral surface of the disc was the most frequent site of maximal degeneration. Only 1 disc showed these changes to occur in the central surface. Cadavers demonstrating maximal degenerative changes of the disc gave no history of temporomandibular joint disturbance prior to death. Moreover, neither edentulism nor the cause of death appeared to be a significant and constant factor relating to the maximal degenerative changes found in the articular disc. Microscopic examination of the discs revealed that the group exhibiting maximal degenerative changes showed the most marked microscopic changes in structure. The severest alteration was represented by evidence of bony trabeculae within the disc. The latter observation is similar to that commonly associated with advanced degenerative arthritis.

31. The Histopathological Reaction of the Gingiva in Hamster Periodontal Disease. *Emmett R. Costich, D.D.S., Ph.D., Eastman Dental Dispensary, Rochester, N. Y.* The jaws of hamsters 30, 45, 111, 260, 365, and 730 days of age were examined histologically with serial sections stained with hematoxylin and eosin and Mallory's. Accumulations of plaque material were seen in the gingival crevice with no breaks or ulcerations observed in the crevicular epithelium. Between the plaque and the epithelium a barrier composed of large numbers of polymorphonuclear leukocytes was seen. No subepithelial reaction such as is seen in human gingivitis was observed in the hamster. Apical migration of the epithelial attachment and alveolar crest resorption occurred with age and to a greater extent in areas of plaque accumulation. The impressions gained from this study were: (1) the polymorphonuclear leukocytes migrate through the intact crevicular epithelium to form a defensive barrier against the irritants in the plaque; (2) the accumulation of debris is a factor in the apical migration of the epithelial attachment and alveolar crest resorption.

32. Punched-Card Recording of Carious Lesions of Both Dentitions of Man. *Gerald J. Cox, Ph.D., and Jessica W. Nathans, A.B., School of Dentistry, University of Pittsburgh, Pittsburgh, Pa.* The method of reduction of the records of dental caries in human teeth to a single punched card of the Hollerith type for each subject, is based on a biserial listing of (1) empirically observed occurrence of carious lesions in (2) a basic arbitrary allocation of areas of carious attack. The number of such arbitrary listings is $2^n - 1$, in which $n = 7$; the empirical number of descriptions depends upon (1) specific teeth and (2) the probable nature of the population from which the subjects are drawn. The records of all 52 teeth of both dentitions are included by use of the principle of recording by position in the arch. Indirectly, the method

deals with caries-free arbitrary areas with no attempt to estimate extent of loss of tooth substance or the nature of repair and, hence, it is applicable only to origin of the lesions rather than to increase in size or to the ultimate fate of the area or of the tooth.

33. Dental Applications of a New Transducer System for Measurement of Pressure Variations in Situ. *W. A. Curby, M.S., Tufts College Cleft Palate Institute, Boston, Mass.* Some of the factors to be considered in the design and construction of a measuring unit capable of recording specific myographic pressures against hard or soft tissue by substances of varying elasticity and hardness are discussed. Part 1 presents some of the factors (anatomic physical-anatomic, physical, chemical, psychologic, and statistical) which must be taken into account when designing any specific pressure evaluation study and gives special requirements for primary and secondary calibration and study of forces under dentures and partials, and between teeth; changes in tongue and lip pressure; and isometric and isotonic measurement of muscle pressure variations. Part 2 gives a description of the design of equipment and establishment of technics which make possible simple measurement and recording of pressure variations. The equipment includes a new resistance pressure element and an impedance matching cathode follower circuit capable of critically damping several types of recording meters differing in full scale deflection characteristics. A description is also given of the modifications necessary to make possible the recording of pressure changes over extended periods of time by means of a resistance controlled pulse generator recording on an Alden 3 channel graphic recorder having single channel timing. The device is small enough to be easily and inconspicuously worn by the individual being tested.

34. Genetics of Primary Tooth Characters. *Albert Dahlberg, D.D.S., Department of Anthropology, University of Chicago, Chicago, Ill.* Certain primary dental characters are unique in genetic studies because of the manner of their occurrence in the dentition. They vary in intensity and in extent of expression and follow the distinct patterns involved in the basic concepts of tooth morphology. Except for the lower incisors the most anterior tooth in each tooth group (incisors, canines, premolars, and molars) expresses characters more intensely than do the teeth distal to it in the same group. Thus, in the molars characters having a genetic basis for existence as distinguished from anomalous and accidental characters are almost always manifest in the second deciduous molar and may be expressed in the first and possibly in the second and third permanent molars as well. This is dependent upon the complex of positive genetic factors calling for the character, on the suppressing factors which operate against expression, but in a reverse order from the third molar forward and in a lesser degree on the environmental factors in development. Peg-shaped second incisors, congenital absence, etc., are results of suppressing factors. These principles are readily demonstrated by the primary characters such as occlusal surface patterns, Carabelli's cusp, the protostylid, etc. Carabelli's cusp, for example, may be present in all the upper molars in one individual, in d m^2 and the first and second permanent molars in another, in d m^2 and the first permanent molars in another, and completely absent in still another. This gives a range of 4 possible degrees of expression aside from consideration of uni- or bilateralism and size of the cusps which would multiply the possible degrees of expression for quantitative determinations.

35. Extraosseous Innervation of the Gingivae. *L. F. Edwards, Ph.D., and Dipya Mongkollugsana, M.S., D.D.M., Department of Anatomy, Ohio State*

University, Columbus, Ohio. Although dental surgeons have known from experience for years that the gingivae are innervated by 2 sets of nerves, extraosseous and intraosseous or alveolar, there is considerable lack of agreement in standard human anatomy textbooks concerning the specific nerves involved. By means of kodachrome illustrations of actual dissections, the extraosseous nerve supply of the gingivae is shown. It was found that the specific nerves involved are the mental, long buccal, lingual, infraorbital, posterior superior alveolar, nasopalatine, and greater palatine.

36. Intracellular Oxidative Enzymes in Oral Tissues. *II.* Succinic Dehydrogenase, DPN Cytochrome C Reductase, Cytochrome Oxidase, and Total Protein in Tongue Mucosa. *Bertram Eichel, D.D.S., and Arnold A. Swanson, M.A., USAF School of Aviation Medicine, Randolph Air Force Base, Randolph Field, Tex.* Prior to an investigation of possible oxidative enzyme changes in the tongue mucosa following x-radiation exposure, it was necessary to establish the presence of certain constituents within the normal tissue homogenate. Total protein, succinic dehydrogenase, DPN cytochrome c reductase, and cytochrome oxidase were quantitatively determined by spectrophotometric analysis. These analyses were simultaneously performed upon submaxillary gland, gingiva, tooth pulp, liver, and brain cortex tissue homogenates derived from each animal, in order to determine the relative concentration levels in the tongue mucosa. A group of 19 normal New Zealand white adult female rabbits were included in this study. The mean total protein content of tongue mucosa was found to be 30 per cent and 20 per cent lower than liver and submaxillary gland, respectively, 20 per cent higher than gingiva and brain cortex, and 70 per cent higher than tooth pulp. The concentration of each enzyme protein was expressed in terms of unit activity. The mean unit succinic dehydrogenase activity was found to be 60 per cent, 40 per cent, and 30 per cent lower than submaxillary gland, brain cortex and liver tissue, respectively; 130 per cent higher than tooth pulp and 410 per cent greater than gingiva. The mean unit DPN cytochrome c reductase activity of the tongue mucosa proved to be 80 per cent and 75 per cent lower than liver and submaxillary gland, respectively, the same as tooth pulp and 400 per cent greater than gingiva. Finally, the cytochrome oxidase mean unit activity of tongue mucosa was 60 per cent and 40 per cent less than submaxillary gland and liver, respectively, 380 per cent greater than tooth pulp and 500 per cent greater than gingiva.

37. Intracellular Oxidative Enzymes in Oral Tissues. III. Spectrophotometric Determination of Pyruvic Reductase (Lactic Dehydrogenase). *Bertram Eichel, D.D.S., Heron E. Pena, and Arnold A. Swanson, M.A., USAF School of Aviation Medicine, Randolph Air Force Base, Randolph Field, Tex.* A study of one of the enzymes involved in glucolysis was conducted in conjunction with the oxidative enzyme evaluation of normal and x-radiated rabbit oral, eye, liver, and brain cortex tissues. The spectrophotometric analysis of the enzyme catalyzed reaction between reduced diphosphopyridine nucleotide (coenzyme 1) and pyruvate with the probable formation of lactate was successfully attempted. The reaction decidedly favors the reduction of pyruvate to lactate and not the oxidation of lactate to pyruvate. The latter reaction proceeds with difficulty in the presence of the oxidized coenzyme (1) under usual conditions. Thus the name 'pyruvic reductase' is applied to the enzyme protein in place of the term 'lactic dehydrogenase.' The reduction of pyruvate behaves in accord with first order reaction kinetics. Straight-line plots have been obtained when activity (the slope of the first order reaction) was plotted

against varying low total protein concentrations (2 to 31 gamma for retina, brain cortex, tongue mucosa, submaxillary gland, and liver tissue homogenates and varying high total protein concentrations), 15 to 425 gamma for tongue mucosa, brain cortex, liver, gingiva, tooth pulp, choroid, submaxillary gland, and lens capsule (ephithelium) tissue homogenates). Comparison of the mean unit enzyme activity (taken as a measure of the enzyme protein concentration) between these tissue homogenates has been made in a group of 12 normal New Zealand white adult female rabbits. Retina homogenates possess 4 times greater mean unit enzyme activity than choroid and liver, 7 times greater activity than tongue mucosa, gingiva, brain cortex, submaxillary gland, and tooth pulp, 20 times greater activity than lens capsule, and 30 times greater activity than lens cortex and lens nucleus tissue homogenates. The probable conclusion can be made that pyruvic reductase is universally distributed in all mammalian tissues.

38. Intracellular Oxidative Enzymes in Oral Tissues. IV. Glucose-6-PO_4 Dehydrogenase in Submaxillary Gland, Gingiva, Tooth Pulp, and Tongue Mucosa. *Bertram Eichel, D.D.S., USAF School of Aviation Medicine, Randolph Air Force Base, Randolph Field, Tex.* Since a marked difference in response for glucose-6-PO_4 dehydrogenase activity was found between retina, choroid, liver, and gingival tissue homogenates obtained from rabbits 24 hours following total head x-radiation, a re-evaluation of the presence of this enzyme's activity in submaxillary gland, tooth pulp, brain cortex, and lens tissue homogenates was undertaken. Whereas the enzyme could be detected with ease in the former tissues, previous attempts to identify it in the latter tissues had met with little success. In the analytical procedure therein employed 0.1 ml. of a 1:50 dilution of each tissue homogenate was used. In the current study, glucose-6-PO_4 dehydrogenase activity could be positively demonstrated spectrophotometrically in all tissues (except lens nucleus) by increasing the homogenate concentration 3 to 5 times, depending upon the tissue studied. The method of assay otherwise remained unchanged. Tongue mucosa was also included in this study since early histologic changes seem to occur within this tissue following x-radiation exposure. The enzyme reactions for all tissues behaved in accord with zero order reaction kinetics. Each of these tissues was studied in a group of 16 normal New Zealand white adult female rabbits. The concentration of the enzyme protein was expressed in terms of tissue unit activity. The mean unit glucose-6-PO_4 dehydrogenase activity of retina was again found to yield the highest value, possessing approximately 4 times greater enzyme content than the tongue mucosa, the second highest tissue. The mean unit enzyme activity for tongue mucosa was only slightly greater than that of choroid, gingiva, and liver, respectively, and approximately 5 times greater than brain cortex and tooth pulp, 10 times greater than submaxillary gland, 15 times greater than lens capsule (epithelium), and 50 times greater than lens cortex. It may be concluded that glucose-6-PO_4 dehydrogenase is virtually universally distributed in all tissues.

39. Psychologic Factors in Adjustment to Full Denture Prosthesis. *William A. Emerson, D.M.D., and Donald R. Giddon, M.A., Tufts College Dental School, Boston, Mass.* An attempt has been made to show what factors other than oral conditions lead to failure of individuals to adjust to full denture prosthesis. Eighty-six clinic patients who had been wearing dentures for periods ranging from 6 months to 20 years were examined and the oral conditions evaluated. The dentures were rated as excellent, average, or poor, on the basis of construction and the existing oral conditions. These subjects were then

asked to fill out a health questionnaire of 43 questions, 32 of which were taken from the Bell Adjustment Inventory (with permission of Hugh M. Bell and Stanford University Press) and were designed to measure the ability to adjust to general health problems. The remaining 11 questions were designed to indicate the subject's attitudes toward dentistry and the dentures being worn. The data from 17 subjects were not included because of oral conditions which made adjustment difficult. The scores from the 2 sets of questions were evaluated using the Phi coefficient. A correlation existed between the degree of satisfaction with the dentures and the patient's ability to adjust to general health problems. The Phi value was equal to 0.37 at a level of significance between 0.05 and 0.01.

40. The Binding of Biologic Amines by Connective Tissue. *Milton B. Engel, D.D.S., M.S., Norman R. Joseph, Ph.D., and Hubert R. Catchpole, Ph.D., Colleges of Dentistry, Pharmacy, and Medicine, University of Illinois, Chicago, Ill.* Connective tissues contain components which behave as negatively charged colloids and which bind bases. These charged aggregates include the glycoprotein-containing ground substance. An electrometric method has been used to study the properties of connective tissue in rabbit skin. Here, X, the normal density of negative colloidal charge, is about 0.07 equivalents per kilogram of tissue. After the application of bivalent cations such as Ca, Mg, and Pb, the charge density is lowered due to combination with the negative groups. In order to determine the combination of connective tissue colloids with organic nitrogen compounds, the following substances have been studied: lysine, arginine, histidine, histamine, tyramine, tryptamine glucosamine, glutamic acid, alanine, protamine, procaine, and thiamine. The amino acids and amines were studied at 0.001 M concentration; protamine sulfate at 2 per cent and procaine hydrochloride at 1 per cent concentration. The basic amino acids, amines, and protamine combined with 30 to 50 per cent of the negative colloidal groups of rabbit skin. Alanine and glutamic acid showed no measurable effect. Ammonium ion at 0.0001 M concentration lowered X by about one-third. Equilibrium constants and free energies of formation and exchange have been computed for these reactions. Inorganic ions, biologic amines, and amino acids, vitamins, hormone precursors, basic proteins, and drugs would appear to take part in exchange with each other, competing for the colloid according to the mass action law and the principle of Le Chatelier.

41. The Catalase Content of Blood From Normal Individuals and Those Suffering From Periodontal Disease. *H. R. Englander, D.D.S., M.P.H., R. Q. Blackwell, Ph.D., and L. S. Fosdick, Ph.D., Great Lakes Naval Training Center and Northwestern University Dental School, Chicago, Ill.* In 1952 Takahara (*Lancet* **52**: 1101, 1952) discovered a new disease, acatalasemia, characterized by the absence of catalase in the blood. The disease occurs primarily in young individuals and is characterized by a gangrenous infection of the gums accompanied by resorption of alveolar bone with early loss of the teeth. Insofar as this condition had been diagnosed as either noma or "pyorrhea alveolaris" by the Japanese dentist, it was thought that there may be some relation between the catalase activity of the blood and severe periodontal disturbances. In view of this the catalase activity was determined on a number of young individuals, recruits of the Great Lakes Naval Training Center and students at the Northwestern University Dental School, and on individuals from the Northwestern University Dental Clinic who were suffering from periodontal disturbances. No readily apparent differences in catalase content of the 2 types of blood were observed.

42. X-Ray Study of the Postulated Development of Fluorapatite During the Fluoride Treatment of Powdered Dental Enamel. *Robert B. Fischer, Ph.D., Charles E. Ring, B.S., and Joseph C. Muhler, D.D.S., Ph.D., Department of Chemistry, Indiana University, Bloomington, Ind.* Fluoride treatment of dental enamel causes a partial replacement of the original apatite structure by a calcium fluoride structure. A revised x-ray diffraction procedure has now been developed to provide x-ray patterns of sufficient resolution to distinguish between hydroxyapatite and fluorapatite. A curved crystal serves to separate highly monochromatic x-rays from the output of a conventional x-ray tube. These monochromatic rays are used in a special self-focusing camera to obtain photographically the desired diffraction pattern. The patterns exhibit, in general, sharper lines and also decreased background intensity, relative to line intensity, than do patterns obtained by more conventional technics. Sodium chloride is used as an internal standard in some of the work. Hydroxyapatite and fluorapatite may be readily distinguished from each other, although small amounts of one in the presence of the other cannot be detected by this procedure. Patterns of dental enamel reveal the hydroxyapatite structure, not the fluorapatite structure, even after fluoride treatment severe enough to bring out the calcium fluoride lines quite intensely. Therefore, fluorapatite is neither a major second product nor a major intermediate product upon the treatment of dental enamel with fluoride reagents.

43. Infrared Absorption Spectra of Dental Enamel and Related Substances. *Robert B. Fischer, Ph.D., Charles E. Ring, B.S., and Joseph C. Muhler, D.D.S., Ph.D., Department of Chemistry, Indiana University, Bloomington, Ind.* The pressed-plate technic of mounting inorganic solids for infrared spectrophotometry has been employed in the study of powdered dental enamel and of related substances. A mull is formed by grinding together the substance to be examined and a diluent which itself exhibits only general absorption of infrared irradiation. For this study, potassium iodide was found to be superior to the more conventional potassium bromide as the diluent. This mull is then pressed into a thin plate for observation in the infrared spectrophotometer. Spectra have been taken of the following: several types of calcium phosphate, including fluorapatite; several forms of calcium carbonate; several types of stannous phosphate; and human dental enamel both before and after treatment with fluoride reagents. Characteristic absorption bands are found for the various polyatomic ionic groupings. The enamel spectrum exhibits characteristic absorptions for phosphate, carbonate, and hydroxyl groupings. The spectrum of the enamel changes considerably upon treatment with some fluoride reagents.

44. The Effects of Certain Antibiotics on Experimentally Induced Caries in Rats. *Robert J. Fitzgerald, Ph.D., and H. V. Jordan, M.S., National Institute of Dental Research, National Institutes of Health, Public Health Service, U. S. Department of Health, Education and Welfare, Bethesda, Md.* In a previous report Stephan et al. (*J. D. Res.* **31**: 421, 1952) described the effects on caries in rats of a number of antibiotics incorporated into Diet 585 on which rats normally showed a high incidence of occlusal fissure lesions. In the present study the same diet and methods were employed to test the following antibiotics at the indicated level per gram of diet: penicillin 10 units, zinc bacitracin 10 units, erythromycin 10 μg, carbomycin 10 μg, and neomycin 10 μg. One hundred forty-four weanling female Sprague-Dawley rats were randomly distributed into 6 groups and housed 3 per cage. Diet and water were available ad libitum. After 90 days on the respective diets, the surviving

rats were scored for caries by the method of Cox et al. (*J. D. Res.* **18**: 469, 1939). The caries results, expressed on a group basis were: penicillin incidence of rats with caries 32 per cent, caries score per rat 0.8; erythromycin, incidence 33 per cent, score 1.9; zinc bacitracin, incidence 87 per cent, score 6.1; carbomycin, incidence 96 per cent, score 10.0; neomycin, incidence 95 per cent, score 13.8; controls, incidence 96 per cent, score 15.8. For all experimental groups with the exception of the neomycin group the caries scores were significantly less than that of the control ($p < 0.01$).

45. Growth and Development of Tooth Germ Homotransplants in Ovariectomized Guinea Pigs. *Harold S. Fleming, D.M.D., D.Sc., School of Medicine, Yale University, New Haven, Conn.* Young adult female guinea pigs were ovariectomized, and in 5 to 10 days transplants were made to the anterior chamber of the eyes of these animals; parallel transplants were also made to normal females. Embryonic tissues (25 to 30 days of age) from a single pregnant guinea pig were used for each series. Approximately 200 guinea pigs plus normal controls have been used. In addition, about 50 animals received brain transplants. Ovariectomized animals receiving transplants were divided into 3 groups. The 1st group received the transplant only. The 2nd group received the transplant impregnated with a methylcholanthrene crystal. Animals in the third group received a 1 mg. intramuscular injection of diethylstilbesterol 3 times weekly. The hosts were sacrificed intermittently and the transplanted tissues recovered and prepared for microscopic study. Results indicated that environmental changes induced in the hosts by removal of ovaries influenced growth, development, and mineralization of tooth germ transplants. Comparison with other studies indicated that size, growth, and development of transplanted tooth germs in untreated ovariectomized animals were the best yet obtained. However, these explants were not as well calcified as those in normal controls. Methylcholanthrene-treated tissues in ovariectomized animals consistently dedifferentiated. It would appear that a complete normal endocrine balance with reference to mineralization of tooth germ transplants cannot be fully restored to ovariectomized animals by diethylstilbesterol injections. Enamel and dentin did, however, appear to reach maturity earlier in those treated with diethylstilbesterol. Vascularity appeared similar in these instances and in the untreated ovariectomized animals.

46. The Inhibition of Smooth Surface Rat Caries by L-Lysine. *J. E. Folk, Ph.D., and F. J. McMcClure, Ph.D., American Dental Association Research Fellowship, and National Institutes of Health, Bethesda, Md.* Smooth surface caries is produced in white rats by diets containing "dry autoclaved" skim milk powders. (*Proc. Soc. Exper. Biol. & Med.* **83**: 21, 1953.) Interest in L-lysine in this regard originates from the fact that the further heat treatment of skim milk powders, brought about by "dry autoclaving," results in a significant loss of available lysine. Diets containing this autoclaved skim milk powder were inadequate for normal growth in white rats. A dietary supplement of L-lysine resulted in an increased rate of growth. A pronounced inhibition of caries was evident at levels of L-lysine monohydrochloride ranging from 0.25 to 2.50 per cent of the diet. Under a regimen of controlled feeding, rats in control and lysine groups had approximately equal rates of growth. At the 0.25 per cent level of L-lysine HCl, caries incidence was reduced from 83.72 per cent and 20.93 per cent and the average caries score was reduced from 5.35 to 0.79. A level of 2.5 per cent L-lysine hydrochloride was only slightly more caries-inhibitory than 0.25 per cent. In addition to a loss in available lysine, the dry autoclaving of skim milk powder also brought about a notable loss in

arginine and histidine. When added to the diet containing the autoclaved skim milk powder, L-arginine as well as L-histidine did not reduce the smooth surface caries produced by this diet. In connection with these experimental rat caries results, basic chemical changes which occur during heat processing of milk powders, especially those contributing to the loss of lysine, are being studied. The manner in which lysine may inhibit caries as produced by this particular diet containing autoclaved skim milk powder is entirely speculative.

47. A Correlation of the Lactate and pH of Dental Plaques of Caries-Active and Caries-Inactive Individuals. *L. S. Fosdick, Ph.D., R. Q. Blackwell, Ph.D., and J. C. Calandra, B.S., Ph.D., M.D., Northwestern University Dental School, Chicago, Ill.* The pH and total lactate content of 400 samples of dental plaques from 100 caries-active cases and 400 samples from 100 caries-immune cases were determined at intervals after a 50 per cent sucrose rinse. It was found that the pH and free lactic acid in the plaque showed a good correlation. The total lactate yields a fair correlation. The acid neutralizing power of the plaques from caries-immune people was considerably less than that from caries-active individuals.

48. A Mathematical Analysis of the Function of the Fibers of the Periodontal Membrane. *Arthur B. Gabel, D.D.S., M.A., School of Dentistry, University of Pennsylvania, Philadelphia, Pa.* A mathematical analysis of the fiber support theory was made. It was found that, based on this theory, the waviness of the fibers has no mechanical justification. The capillary blood volume of the membrane was calculated for a model tooth with a membrane 250 μ thick. The volume displaced by the root when the same tooth was displaced axially 25 μ was also calculated. These volumes are practically equal. For this displacement the necessary elastic elongation of the fibers was 4 per cent, so that if their waviness equaled or exceeded that amount the membrane would be under compression and the fibers would be under little or no tension. Synge showed that a thin incompressible membrane would be squeezed tangentially along the root surface but would offer great resistance to the tooth approaching the bone. He also showed that the displacement normal to the root surface would be only a fraction of that tangent to it. Using that fact, we found that for the same model tooth the fibers would be put under tension by the pressure within the membrane and would function in the same manner as the cables of a suspension bridge, thus increasing the load required to produce a given displacement of the tooth. The tensions in the fibers were calculated for various degrees of waviness. We found that, for the model tooth, a waviness even in excess of 4 per cent would permit the fibers to be under strong tension.

49. The Effect of Endocrines on the Eruption Rate of the Upper Incisor of the Rat. *Leonard David Garren, D.M.D., Harvard School of Dental Medicine, Boston, Mass.* Doses of cortisone of 1, 2.5, 5, and 10 mg. per day were injected subcutaneously into 50 male rats beginning at 80 days of age and continuing for 4 weeks. The weekly eruption rate of the upper incisor in these and appropriate control animals was measured using the technic of Schour. All doses of cortisone caused the eruption rate to increase significantly within 2 weeks. No strict proportionality between dose and response existed, but the response tended to increase with increasing dosage up to 5 mg. per day, which produced a 28 per cent increase in eruption rate. The response to 10 mg. per day was not significantly different. Histologic and histochemical sections of the incisors did not demonstrate changes sufficient to account for the results. Pre-

liminary experiments indicate that the eruption rate is reduced following bilateral adrenalectomy and is returned to normal by the injection of cortisone. Desoxycorticosterone acetate did not appear to have a significant effect on the eruption rate of either normal or adrenalectomized animals. Another study indicated that the decreased incisor eruption rate in hypophysectomized animals can be significantly increased by the injection of cortisone. In line with the work of Becks et al. (1954), a relationship between thyroid activity and eruption rate has been demonstrated. The feeding of 0.5 per cent desiccated thyroid mixed with ground rat chow caused a 30 per cent increase in the incisor eruption rate in 80-day-old male rats after 2 weeks. The eruption rate subsequently decreased toward normal, although thyroid feeding was continued. The feeding of a diet containing 0.15 per cent propylthiouracil mixed with ground rat chow slowed the incisor eruption rate; by the 4th week the decrease reached 48 per cent of that observed in the control group.

50. Sensory Responses Following Electronic Stimulation of Oral Tissues. Part III. The Thalamus. *Joseph A. Gibilisco, D.D.S., M.S., USAF School of Aviation Medicine, Randolph Air Force Base, Tex.* Nervous activity of central nervous system centers associated with trigeminal conduction is being investigated. Those tissues innervated by peripheral branches of the 5th nerve have been stimulated in the cat. The resulting excitation has been recorded in the gasserian ganglion and the medulla. The data collected from these areas have been previously presented. This report is primarily concerned with thalamic activity, although nervous activity in other brain areas associated with trigeminal excitation is presented. A stereotaxic instrument is adapted to the anesthetized animal. Fixed coordinates permit 4 electrodes to probe the thalamic nuclei. Various electrical potentials are applied to the oral tissues. These result in nerve excitation which is conducted to the thalamus. A 6-channel ink-writer and cathode ray oscilloscope records activity from all the electrodes. Spontaneous thalamic activity, sleep patterns, and anesthetic potentials are reduced with deep anesthesia. This permits better observation of the characteristics of the trigeminal modalities. When the gingiva or tooth pulp are stimulated an action potential results. This consists of an initial artifact followed by spikes indicating the type of the sensation. Pain and temperature predominate. Stimuli applied to the 5th nerve successfully excite the thalamus. Following each experiment, the brain is removed and microscopic sections are prepared. The location of the electrodes is then indicated by the stain pattern. The technic lends itself to numerous possibilities for studying nerve function.

51. Statistical Evaluation of Multivariate Data in Dental Research. *Donald B. Giddon, M.A., and Vincent F. Lisanti, D.M.D., Tufts College Dental School, Boston, Mass.* A more efficient approach has been developed to analyze massive amounts of clinical and biochemical data for possible correlation. By the use of nonparametric correlative technics such as the Phi contingency coefficients, it is possible to screen out promising relationships without immediate concern as to the normality of the distribution homoskedasticity, number of cases, etc. This method is of particular value with most clinical variables where the scores or ratings are not in quantitated intervals. In such cases it becomes a fairly easy matter to handle the clinical variable as dichotomous. The quantitated variable, such as an enzyme titer, is then artificially dichotomized with respect to the median. A rapid statistical operation will reveal the significance of the relationship. All variables which are related at a probability of less than 0.10 are carefully analyzed for form of distribution homoskedasticity,

linearity, and number of cases. If the assumptions underlying the more sensitive parametric correlative technic can be met, more powerful statistics may be employed. Certain transformations of the data may be allowed in order to correct for skewness and other irregularities of the distribution, but only if the underlying assumptions are fully understood. Utilizing transformations, it may also be possible to increase the size of the sample by including data from previous studies collected under similar conditions. The ultimate aim of this approach is to provide a sound economical means for ascertaining the relative prognostic significance of a large number of variables either singly or in combinations with each other. Additional statistical procedures are discussed for weighting various combinations of variables.

52. Differences in Bone Resorption Between Normal and Ia Implants. *Roy Gillette, Ph.D., Dorothy Mardfin, A.B., and Isaac Schour, Ph.D., D.D.S., University of Illinois, College of Dentistry, Chicago, Ill.* The method of transplantation was used to determine whether the deficiency in bone resorption which characterizes the *ia* rat is a defect in the bone tissue itself or in the endocrine environment controlling bone resorption. An inbred strain of rats was used, in which abnormal animals can be distinguished from their normal littermates 20 days after birth by their failure to erupt the incisors (*ia* condition). In endochondral bones the excessive lengths of the primary trabeculae of the metaphyses are indicative of the retardation in bone resorption characteristic of the *ia* rat. In 144 transplantations involving 96 animals the chondrocostal growth centers of normal and *ia* donors 36 days of age were removed and implanted subcutaneously into *ia* littermate hosts and also into normal littermate hosts. Implants were recovered after 7, 14, 21, and 28 days. The growth centers either became well vascularized and produced trabeculae of endochondral bone or degenerated due to incompatability with the host. The lengths of the primary trabeculae of successful 28-day implants were measured. In *ia* hosts the trabeculae of normal implants were one-half the length of those produced by *ia* implants. Thus, in spite of their location in the *ia* host, the tissues of the normal implant materially influenced the resorption of the bone produced. Our findings suggest that the resorption deficiency in the *ia* rat is related to tissue factors as well as to the hormonal factor which had been previously proposed.

53. The Effect of Sodium Dehydroacetate on Growth and Glycolysis. *Robert E. Gillis, D.D.S., Department of Microbiology, School of Medicine, Western Reserve University, Cleveland, Ohio.* Sodium dehydroacetate has been shown to have a wide spectrum of antimicrobial activity. It has been suggested that this compound inhibits acid production in the dental plaque by virtue of its effect on the enzymes of the classical Embden-Meyerhof-Parnas pathway of anaerobic glycolysis. Experiments were devised to test the effect of sodium dehydroacetate on the growth and glycolysis of *Lactobacillus casei* (A.T.C. 4646). The effect of this compound on the glycolytic system of an extract of baker's yeast was also tested for comparative purposes. It was found that 0.01 M sodium dehydroacetate markedly inhibited the growth of *Lactobacillus casei* in a medium of maximal nutrient value. In experiments with resting cells of this organism, sodium dehydroacetate in concentrations of 0.01 M to 0.05 M failed to inhibit either the rate or amount of acid production from glucose. In similar experiments with a macerated yeast juice, no inhibition of glycolysis was observed with 0.01 M sodium dehydroacetate. When the concentration was raised to 0.05 M, a slight inhibition was obtained. There is abundant evidence that the Embden-Meyerhof-Parnas scheme is a major glycolytic mechanism in yeast, and we have recently shown it to be a major

pathway in *Lactobacillus casei*. Since glycolysis is not markedly inhibited by 5 times the concentration of sodium dehydroacetate which inhibits growth, it is concluded that the primary site of action of this compound is not on an enzyme of the Embden-Meyerhof-Parnas pathway.

54. A Method to Study the Role of Saliva in the Carious Process. *Paul W. Goaz, D.D.S., Oklahoma Medical Research Foundation, Oklahoma City, Okla.* To study the role of saliva in the carious process a method for assaying the influence of the saliva on the decalcifying activity of lactobacillus was developed: saliva was sterilized with ethylene oxide using a modification of the technic described for sterilizing bacteriologic media. The sterilized saliva was incubated with powdered tooth substance and an oral lactobacillus culture suspended in a glucose solution. The amount of soluble calcium formed was taken as the end point. When the saliva was sterilized with ethylene oxide, the calcium values obtained were 2 to 4 times greater than with autoclaved saliva and 2 to 6 times greater than with Seitz-filtered saliva. To demonstrate an application of this technic, salivas from 5 patients with endocrine disorders were assayed during therapy. The administration of increasing amounts of thyroxin to 2 hypothyroid patients was reflected in the increased decalcifying activity of the test microorganisms incubated in their saliva. Radioiodine and thyroidectomy produced a change in the saliva from 2 hyperthyroid patients that was manifested by a decrease in the decalcifying activity of the microorganisms. The onset of puberty following chorionic gonadotropin therapy in a 15-year-old boy with delayed adolescence was accompanied by an increase of this activity. Continued study of the factors involved in this approach to the problem is indicated and may illustrate that the etiology of caries is not entirely oral in origin, but that systemic factors are influential by their physiologic control of the composition of saliva.

55. Periodontal Disease in Syrian Hamsters. *Harold S. Gold, A.B., and Paul H. Keyes, D.D.S., M.S., Harvard School of Dental Medicine, Boston, Mass.* Spontaneous periodontal disease has been observed to occur under a wide variety of conditions and to a highly variable extent in a collection of over 1,000 hamster jaws. The lesions have been associated with soft subgingival accumulations as well as hard calcareous deposits. In some instances alveolar bone resorption has been so extensive that involved teeth appear to be near exfoliation. To determine whether a relationship exists between the sex of the animal and susceptibility to periodontal disease, a comparison of alveolar bone resorption was made between 20 male and 20 female hamsters raised and maintained on a purified ration (Shaw, *J. D. Res.* **26**: 47, 1947) from birth to 121 days. The maxillary bone loss in the females was significantly greater ($p = 0.02$). To study the possible effect of weanling ration on susceptibility to periodontal disease, a comparison was made between 2 groups of male hamsters. One group of 15 was born and raised for 21 days on Purina Laboratory Chow, and then transferred to a test cariogenic diet (Keyes and Schourie, *J. D. Res.* **28**: 138, 1949) for 100 days. The other group, numbering 12 animals, was raised on the cariogenic diet and continued thereon until 121 days of age. Maxillary bone loss was significantly greater ($p > 0.01$) in those animals exposed continuously to test diet.

56. The Measurement of Blood Loss During Operation for Multiple Extraction and Alveoloplasty. *Robert J. Gores, D.D.S., Section of Dentistry, Mayo Clinic, Rochester, Minn.* The photelometric method, an accurate method for the measurement of blood loss in oral surgical procedures, was used. It is

based upon the quantity of hemoglobin lost due to the bleeding which occurs during surgery. A general anesthetic was employed in all cases studied. Data are presented to show that blood loss during operation for multiple tooth removal and alveoloplasty is not excessive in most cases. (By excessive blood loss we mean more than 500 c.c. of blood.) However, in a few cases the quantity of blood lost during this type of surgery was found to be equal to some general surgical procedures such as gastric resection of simple mastectomy. In addition, data are also presented to show that by the local use of a vasoconstricting agent, even in very small concentrations, the average blood loss may be reduced by as much as 50% when compared with those cases in which a vasoconstricting agent is not used.

57. Exact Analysis (by Binomial Summation) of Fourfold Contingency Tables Arising From Recombination Experiments With Fusospirochetal Exudate. *R. M. Grainger, D.D.S., M.Sc., D.P.H., and J. B. MacDonald, D.D.S., Ph.D., Division of Dental Research, Faculty of Dentistry, University of Toronto, Toronto, Canada.* This report covers the selection of appropriate statistical devices for testing the hypotheses entailed in experiments set up to discover a minimum recombination of organisms capable of producing typical lesions in guinea pigs. Economy in animals and laboratory time were made possible by use of the exact methods when application of the conventional normal curve approximation and the chi square test were invalidated by the wide deviation of the hypothetical proportion from the mid-value of 0.5 and by small numbers of animals.

58. Cellular Response to Metallic and Nonmetallic Materials in Vitro. *Nicholas G. Grand, D.D.S., M.S., School of Dentistry, St. Louis University, St. Louis, Mo.* Owing to the interest in the cellular response of the host to dental and surgical materials, and the difficulty of controlled evaluation of the action of these materials in vivo, we have undertaken a relatively simple in vitro assay using tissue cultures. These in vitro studies of cellular response to dental and surgical materials utilize lymphoid tissue which gives a chemotactic as well as fibroblastic response. A grading of chemotactic response of ameboid cells and fibroblasts in tissue culture to the test materials and the effect of these materials on cellular proliferation and migration will be presented. Among the dental restorative materials there is a wide spectrum of cellular response; for instance, silver points induce positive chemotaxis with fibroblastic proliferation, while gutta percha points induce an indifferent chemotactic response but are inhibitory to fibroblastic proliferation. The implication of the data presented relative to the use of these materials in dentistry and surgery will be discussed.

59. Inhibition of Oral Lactobacilli by Streptococci. *Gordon E. Green, Ph.D., and Helen S. Inverso, M.S., Dental Research Laboratory, Ohio State University, Columbus, Ohio.* Previous reports indicated an association of mucoid streptococci with caries activity and nonmucoid strains with caries inactivity. Studies were made of factors concerned with demonstration of inhibition of lactobacilli in vitro. It was found that streptococcus cultures should be freshly isolated, while the age of lactobacillus cultures was not important. Using 24-hour brain-heart infusion broth cultures, lactobacilli were seeded into brain-heart infusion agar plates, and streptococcus cultures were tested by placing a drop on 1 quadrant of the plate. Eighty-six strains of mucoid and nonmucoid streptococci isolated from human salivas were tested against 10 strains of lactobacilli similarly isolated. Species identifica-

tion of the streptococci showed that most were *S. mitis* or *S. salivarius*. No difference between streptococci isolated from caries-immunes and caries susceptibles was seen. Intermediate colony types of lactobacilli were apparently more resistant to inhibition than were smooth or rough types. There was no difference in the inhibitory capacity of the streptococcus species with respect to various colony types of lactobacilli. Sixty-one per cent of the nonmucoid mitis strains and 47% of the mucoid salivarius strains inhibited growth of lactobacilli, while 55% of the mitis strains inhibited acid production by lactobacilli and only 11% of the salivarius strains had this capacity. Quantitative examinations of immune and susceptible salivas for these streptococcus species were made. The results indicate that the predominance of mucoid or nonmucoid strains has no relation to the number of possible inhibitory organisms present.

60. Correlation of Clinical Diagnosis and Histopathologic Findings in 101 Pulpless Teeth With Areas of Rarefaction. *Louis I. Grossman, D.D.S., D.M.D., and Samuel R. Rossman, D.D.S., School of Dentistry, University of Pennsylvania, Philadelphia, Pa.* In cases where root resection was done, periapical tissue, with or without root tip, was sent to the Oral Pathology laboratory. The clinical diagnosis had been previously established by a member of the Endodontic department, but the person examining the microscopic material had no knowledge of the diagnosis. By this blind test method, 101 consecutive specimens have been examined with the following results: 56.3% of 32 cysts as determined microscopically were interpreted correctly from x-ray examination, and 43.7% incorrectly. Of 69 granulomas or chronic abscesses, the clinical diagnosis was correctly established by x-ray in 72.5% of cases, and incorrectly in 27.5%. These values differ significantly from those reported by Priebe et al. (*Oral Surg., Oral Med. & Oral Path.* **7**: 979, 1954), but tend to show that cysts are more often present than is possible to determine by x-ray examination alone.

61. A New Experimental Animal for Periodontal Studies. *O. P. Gupta, B.D.S., M.Sc., and James H. Shaw, Ph.D., Harvard School of Dental Medicine, Boston, Mass.* In the search for experimental animals which are susceptible to periodontal disease, 8 rodent species previously uncommon in laboratory studies were examined: red-backed mouse, meadow vole, white-footed mouse, giant mouse, wood rat, Chinese hamster, rice rat, and Mongolian gerbil. The specimens used had not been specially prepared by laboratory maintenance. The rice rat (*Oryzomys palustris*) showed typical periodontal lesions. Detailed microscopic, roentgenographic, and histologic studies of the soft and hard oral tissues of 8 rice rats (12 to 37 months old) were made. Extensive packing of hair between the teeth and gingivae and marked recession and atrophy of gingival tissue were noted. Radiographs revealed marked resorption of alveolar bone and rarefied areas at the periapical region. In some cases little bone was left and the teeth were loose. Slight calculus occurred on the molar crowns but was not found near or beneath the gingiva. Two 25-day-old rice rats already had detectable lesions. In a preliminary experiment the effect of 3 diets, laboratory chow, a grain diet, and a purified ration (Shaw, *J. D. Res.* **26**: 47, 1947), was studied for the incidence of periodontal disease. The rice rats grew normally and were maintained in good health on each diet. Clinical examination of 14 rats after 9 weeks showed 3 without evidence of periodontal disease, 6 with early lesions, and 5 with large lesions. Since this species can be maintained in the laboratory on commonly available diets, it promises to be a valuable adjunct to periodontal disease research.

62. Microradiographic Study of "White Spots" in the Enamel. *Cesar Guzman, D.D.S., Herbert Mermagen, B.S., and Finn Brudevold, D.D.S., M.S., Eastman Dental Dispensary and Atomic Energy Project, University of Rochester, Rochester, N. Y.* Ground sections from teeth with "white spots" in the enamel were studied by means of soft-x-rays. By using a very fine-grained photographic emulsion, it was possible to make a microscopic study of the radiographic image under magnifications over 500 times. The white spots appeared as radiolucent areas. While demineralization frequently was extensive in the subsurface enamel, the surface enamel external to the lesion appeared to be normal with a radiopacity similar to that of sound enamel. Demineralization seemed to progress along 2 pathways: 1. Inward along the interprismatic substance. This was suggested by a widening of the radiolucent areas between the prisms. 2. Laterally along the striae of Retzius. Within the lesion, the striae of Retzius appeared as lines of high radiolucency, increasing their thickness gradually in several segments. This radiolucency always extended toward the adjacent peripheral striae. The radiographic image of the lesion was always smaller than that observed by transmitted light examination.

63. In Vivo pH Determinations on Carious and Noncarious Teeth in the Albino Rat. *John Haldi, Ph.D., Winfrey Wynn, M.A., Katherine Bentley, A.B., and Mary Louise Law, A.B., Division of Basic Sciences in the Health Services, Emory University, Emory University, Ga.* Numerous pH determinations on rats' teeth have been made under various experimental conditions with a very small antimony electrode. In 1 experiment a total of 1,820 readings were taken at different times on specific surfaces of the teeth irrespective of the presence or absence of caries. A pH below 6 was obtained in only 111 readings with only 34 as low as or lower than 5.5. Any correlation that may be found between the caries score and the pH readings will be reported. In another experiment 0.8 c.c. of a 10% sucrose solution was placed in the mouths of 12 animals and readings taken at 0-, 2-, 5-, and 15-minute intervals. Only 1 of 384 readings was below 6.0. As caries in the albino rat is usually initiated in the fissures and as it was impractical to construct an electrode small enough to reach the bottom of the fissure, other experiments were conducted in which holes were drilled in the teeth at the beginning of the experiment large enough to accept the electrode used previously. pH readings were taken in these cavities at intervals until they became carious. Toward the end of the experiment a number of readings of 5.5 or lower were obtained. It is impossible to state from these preliminary experiments whether the low pH readings occur prior or subsequent to the onset of caries.

64. Comparison of the Tissue Response to Rotary and Ultrasonic Dental Cutting Procedures. *Louis S. Hansen and Arne G. Nielsen, U. S. Naval Dental School, National Naval Medical Center, Bethesda, Md.* One of the 4 incisor teeth in each of 40 guinea pigs was cut with an ultrasonic dental cutting instrument developed at the National Naval Medical Center; 1 other was cut with a conventional rotary instrument; and the remaining 2 were left uncut for controls. After a 2-week postoperative period the jaws of the animals were removed and examined microscopically. Eighty-three of 142 specimens suitable for evaluation revealed no significant alterations from the normal. Sixty-one of the 83 were controls, and 22 were from the rotary group. No teeth cut by the ultrasonic process were without significant deviations. Thirty-four specimens exhibited a minimal disturbance in amelogenesis. Of these, 12 were found in each of the rotary and control groups, and 10 in the

ultrasonic. Six specimens revealed a severe disturbance in amelogenesis. All 6 were in teeth cut by the ultrasonic process. Nine specimens, all of which had been cut by the ultrasonic method, exhibited severe interruptions of amelogenesis and dentinogenesis. Marked osteodentin formation was a feature of this group. In 10 specimens, also cut by the ultrasonic method, amelogenesis and dentinogenesis were so severely disturbed that enamel and dentin formation had ceased. Replacement of tooth-forming elements with fibrous connective tissue was apparent. In the incisor teeth of guinea pigs more severe and more frequent disturbances in amelogenesis were found to be associated with the ultrasonic vibratory cutting process than with conventional methods. Severe pulp changes and alterations in dentin formation, which were not present in teeth prepared by rotary methods, were found in teeth cut by the ultrasonic handpiece.

65. Systems for Processing Data on Biologic Activity of Numerous Chemicals. *G. L. Hargreaves, M.S., and R. S. Manly, Ph.D., Tufts College Dental School, Boston, Mass.* Three general systems have been developed and used for locating, sorting, and correlating information regarding the inhibitory effect of a large number of chemicals on salivary sediment. More complicated systems become efficient as the number of chemicals increase. At first 2 simple card files were sufficient for listing the compounds by code and chemical name to permit checking whether compounds had been previously tested and what the results were. When 700 to 1,000 chemicals had been studied, a change was needed to make searching efficient and to ease the problem of correlating chemical structure with biologic activity. A 5 by 8 "E-Z" sort card with edges punched was found suitable for recording the code letters, structural formula, test data, the name of the compound, the supplier number, the date of test, and the data regarding findings. At approximately 1,500 chemicals the edge-punched cards became unwieldy and had to be supplemented by an IBM punch card using the Wyandotte chemical classification code, involving also a recording of most of the items found on the 5 by 8 edge-punched cards. Information will be given regarding the practical utility of these 3 systems for different purposes.

66. The Effect of Edta and Food Consumption on Dental Caries in Rats. *M. Rachel Harris, M.S., National Institute of Dental Research, Bethesda, Md.* In previous rat experiments, the addition of EDTA (ethylenediaminetetraacetic acid) to Diet 580 (32% dried skim milk, 66% sucrose, and 2% Wilson's whole dried liver substance) resulted in a highly significant increase in caries and a highly significant decrease in growth. This experiment was set up to determine which of these effects may have been the direct result of EDTA consumption and which may have been an indirect result, due to reduced food consumption. Fifteen litters of 3 Sprague-Dawley female weanling rats were fed (1) Diet 580 plus 0.2% EDTA, ad libitum, (2) Diet 580, pair-fed controls for (1), and (3) Diet 580, ad libitum-fed controls. The animals were maintained on the experiment for 56 days or as long as all 3 animals in a given litter survived (average of 41 days). The average daily food consumption for Group 1 was 2.9 Gm., Group 2 2.9 Gm., and Group 3 6.6 Gm. The average daily weight gains in grams were (1) –0.04, (2) 0.22, and (3) 1.62. The number of animals with caries in each group was (1) 14, (2) 2, and (3) 11; the average number of carious teeth per rat (1) 8.67, (2) 0.20, and (3) 3.07; the average number of carious lesions per rat (1) 22.07, (2) 0.20, and (3) 5.53. The development of caries was significantly higher in the group receiving EDTA than in either control group, and higher in the ad libitum-fed controls

than in the pair-fed controls. These results show that decreased food consumption per se was largely responsible for the decrease in growth, but was not responsible for the increase in the development of caries.

67. Some of the Variables Associated With in Situ Plaque pH Measurements. *John W. Hein, D.M.D., Ph.D., School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* The pH of 5 tooth surfaces in 10 subjects, not brushing their teeth, was determined before and at 5 10-minute intervals after various rinses. The sites measured in the right maxillary quadrant were: adjacent to labial interproximal area between 2-3 and 3-4; labial surfaces of 3 and 4; adjacent to palatal interproximal area between 4 and 5. The following variables were found important: duration of rinse, volume of rinse, carbohydrate concentration, site of measurement, pH of rinse, initial pH of plaque, number of days from cessation of toothbrushing, and type of carbohydrate. The following results were obtained: varying concentration from 1%, 10%, 40%, and 75% increased duration of pH falls, labial interproximal areas fell slightly lower than labial surfaces, palatal area at all times was highest, 30-second rinses gave less prolonged effect than 2-minute rinses, the pH minimum fell rapidly during the first week after cessation of toothbrushing and slightly for the next 2 weeks, small volumes (5 ml.) gave less prolonged pH fall than 25 ml. rinses, 10% soluble starch fell slower but as far as a 10% glucose rinse. The relation of these variables to habits of eating must be noted in comparisons with the ability of various foodstuffs to produce acid in plaques, as shown by the fact that drinking 180 ml. of a soft drink from a bottle gave much higher pH readings than a rinse of 25 ml. of the same material for 2 minutes.

68. Extraction Procedure and Some Characteristics of a Biologically Active Salivary Fraction. *T. J. Hill, D.D.S., M. M. Matt, D.D.S., and S. Pearlman, D.D.S., School of Dentistry and Institute of Pathology, Western Reserve University, Cleveland, Ohio.* The extraction procedure of Birnkrant and Shapiro for a beef parotid gland hyperglycemic factor was adapted to saliva, and, as previously reported, a fraction was obtained which produced hyperglycemia in rats and inhibited the growth of *L. acidophilus* 4646 in vitro. Fresh saliva is centrifuged and $\frac{3}{8}$ volume of 0.1 N sodium hydroxide added to the supernatant to a pH of 11.4 to 11.5. After 18 hours of refrigeration acidify the solution with glacial acetic acid to pH 5.0. Add 60 ml. of warm benzoic acid (30 Gm. benzoic acid in 60 ml. of 95% ethanol) dropwise with vigorous stirring for each liter of salivary solution. After 24 hours of refrigeration, filter, wash precipitate with alcohol, ether, and dry (Ppt. I). Triturate Ppt. I with H_2O (12 ml. per gram). Dialyze for 72 hours at 40° C. Change dialysis water daily. Centrifuge contents of Visking casing and filter. Pervaporate supernatant to small volume in fresh casing. Add absolute alcohol to produce final concentration of 95%. After overnight refrigeration, centrifuge, discard supernatant. Wash residue with alcohol, ether, and dry (Ppt. II). This is the active fraction, a white (sometimes brown) amorphous powder. The material has been kept under vacuum at room temperature for months with no apparent loss of activity. It is water soluble (1 to 2 parts per 100), and insoluble in alcohol concentrations over 40%. Electrophoretic analysis reveals 3 moving components in relative proportions of 60%, 35%, and 5%. Chemically, faintly positive biuret and tyrosine reactions were obtained. Tests for reducing sugars were negative. A fine precipitate is given with 5% trichloracetic acid. In summary, the saliva fraction may be a polypeptide, stable over a broad range of pH.

69. Variations in Salivary Phosphatase Activity. *K. C. Hoerman, D.D.S., M.S., H. R. Englander, D.D.S., M.P.H., and I. L. Shklair, Ph.D., Naval Dental Research Facility, Great Lakes, Ill.* The presence of acid and alkaline phosphatase activity in human saliva has been adequately demonstrated. However, the primary source and specific action there have not been determined. Some information might be elicited from subjecting the fluid to various physical conditions. Twenty-five milliliters of paraffin-stimulated saliva was collected from each of 30 normal male Naval personnel, aged 17 to 29 years. Each fresh sample was tested for enzyme activity in both whole and supernatant states. The remaining sample was divided into 7 aliquots and frozen at –40° C. Fifteen salivas were frozen whole and 15 supernatant. The frozen aliquots were tested subsequently at intervals up to 10 weeks. Phosphatase activity was determined by a modification of the method described by Chauncey et al. Whole fresh saliva exhibited an alkaline phosphatase activity approximately 50% greater than supernatant fresh saliva; at the same time acid phosphatase activity in whole saliva was found to be approximately 60% greater than that in supernatant saliva. In the frozen samples, the alkaline phosphatase activity in both supernatant and whole phases was variable; ranging in increases up to 80% and in decreases to 46%. Acid phosphatase activity of whole and supernatant frozen salivas increased by 35 and 38 per cent, respectively. The findings indicate, in the case of acid phosphatase activity, an enzyme source in the saliva based upon the cell-destructive action of freezing.

70. Use of Repository Tubocurarine to Withhold Pain, Edema, and Spasm Following Surgical Removal of Impacted Mandibular Third Molars. *Murray M. Hoffman, S., D.D.S., Chicago, Ill.* Intramuscular injections, at the time of surgery, of repository tubocurarine (Tubadil) in 264 adult patients who underwent surgical removal of impacted mandibular third molars were effective in significantly withholding pain, swelling, and trismus, the common sequelae attending the unavoidable surgically induced trauma. The dosage was approximately 0.5 mg. per kilogram. The single injection 1.5 c.c. of repository tubocurarine suspension contained 37.5 mg. of tubocurarine. Forty-eight additional patients served as controls. All patients received 600,000 units of intramuscular penicillin suspension and all wounds were sutured per primim. No respiratory depression, diplopia, or other serious side effects from the repository tubocurarine were encountered. Eighty-five per cent of control patients required postoperative analgesics for pain as compared with only 10% in the tested group. The relaxing agent was effective in withholding spasm of the jaw and facial muscles in 92% of the tested cases, whereas 60% of the control group did not manifest varied degrees of trismus. Postoperative edema was prevented in 78% of the patients who received the repository tubocurarine injections whereas the control group showed no swelling or edema in only 17%. The office use of repository tubocurarine in oral surgery is attended without danger or deleterious effect.

71. An Anatomical Study of the Masticatory Space. *Gordon V. Holahan, D.D.S., School of Dentistry, Georgetown University, Washington, D. C.* This fascial space which is commonly involved in dental infections has not been completely described and illustrated in current texts. This study utilized several cadaver heads, one of which was dissected from the lateral approach after the arterial and venous systems had been injected with red and blue liquid latex. The nerves were colored by hand as they were identified and

Kodachrome slides made of each stage of the dissection. Three half heads were frozen in solid carbon dioxide and serial sections made 1 cm. wide in each of 3 planes, coronal, sagittal, and horizontal. The same procedure was followed on 3 more half heads after the masticator space had been injected with yellow liquid latex. Kodachrome slides were made of pertinent sections allowing a comparison of the injected and uninjected specimens in reference to the spread of fluid when infection of the area is present.

72. A Study of the Mechanism Concerned in the Deposition of Lime Salts in Bridging Over A Pulp Exposure. *H. A. Hunter, D.D.S., M.Sc., Faculty of Dentistry, University of Toronto, Toronto, Canada.* Calcific bridging of certain cases of pulp exposure occurs following the empiric use of certain capping materials; and calcium deposition is often seen in situations where there is tissue degeneration associated with fatty acids. This study has investigated the possibility of the latter effect being significant in pulp capping. Using fresh pulp exposures in 42 dogs' teeth, the effects of calcium hydroxide, magnesium hydroxide, zinc oxide–eugenol cement, zinc oxide–phosphoric acid cement, calcium oleate, and cholesterol were observed for periods up to 9 weeks. No evidence of calcific bridging was seen in 7 of the 12 calcium oleate-treated teeth, the other 5 being necrotic; neither was there any bridging in 2 of the 3 cholesterol-treated teeth, the third being necrotic; nor in either of the 2 zinc oxide–phosphoric acid cement-treated teeth. Two of the 5 zinc oxide–eugenol-treated teeth showed depositions about dentin fragments, 1 other showed extensive dentin bridging, and the remaining 2 showed none. Of the 10 calcium hydroxide-treated teeth 6 showed bridging, the remaining 4 were destroyed by suppuration. Five of the 10 magnesium hydroxide-treated cases showed bridging, the other 5 failed because of intervening suppuration. Tests indicated that a bacteriostatic action of calcium hydroxide and magnesium hydroxide was not significant. The similar dentin-stimulating effect of both calcium and magnesium hydroxide may be due to their having an elevated pH. The cation appears to be unimportant so long as it remains bland.

73. Odontiasis in the Monkey Between Birth and Two Years of Age. *V. O. Hurme, D.M.D., and G. van Wagenen, Ph.D., Forsyth Dental Infirmary for Children, Boston, Mass., and School of Medicine, Yale University, New Haven, Conn.* Utilizing analytic technics developed earlier for the computation of standards of variation in odontiasis (*Proc. Am. Philosophical Soc.* **97**: 291, 1953), it was possible to examine longitudinal data on 66 macaques in considerable detail on both individual and group basis. A clear-cut sex difference in the emergence of permanent first molars was discovered. Evidence was obtained of moderate to moderately high positive correlations between the eruption of these teeth and the entire deciduous dentition. Analysis of variations reveals a certain constancy in the operation of the mechanism that determines the schedule of initiation of the process for various teeth. Although this underlying growth mechanism functions at different rates in most monkeys, observable patterns of emergence can furnish useful clues for estimating chronologic ages of young animals with no birth records.

74. Studies of the Microflora of Dental Plaques After Dispersion by Ultrasound. *Helen S. Inverso, M.S., Gordon E. Green, Ph.D., and Matt C. Dodd, Ph.D., Dental Research Laboratory and Department of Bacteriology, Ohio State University, Columbus, Ohio.* Mechanical devices such as mortars have

been used to emulsify plaques; results by such means vary. Reproducible results are obtainable when ultrasonic energy is used to disperse other biologic materials; therefore, this technic was employed for dispersal of plaque material. Plaque material is defined as that remaining adherent to interproximal and cervical areas after forceful rinsing with a water syringe. Energy at a level of 125 milliamperes was supplied by a Brush piezo-electric ultrasonic generator operated by Battelle Memorial Institute of Columbus, Ohio. Time intervals ranged from 2 to 20 minutes; treatment for 3 to 5 minutes in saline solution or in saline plus Tween 80 gave best results. The following observations were made: Counts for all species of microorganisms recovered were higher when plaques were dispersed by ultrasound than when comparable plaques from the same individual were ground in a mortar. Number of microorganisms in plaques taken from the same individual vary greatly. Good correlation was obtained between salivary and plaque lactobacillus counts from the same individuals. Quantitative examinations of microflora from caries-susceptible and caries-immune plaques have been made.

75. Stress Lines Formed in the Class Two Restorations as a Result of Different Cavity Forms. *Ralph L. Ireland, D.D.S., Donald C. Haack, M.S., and Willard F. Guard, D.D.S., University of Nebraska, College of Dentistry, Lincoln, Neb.* Mesiodistal cross sections of Class 2 cavity preparations with flat pulpal walls, flat axial walls, rounded and sloped pulpal walls, rounded and sloped axial walls, square pulpal-axial line angles, rounded pulpal-axial line angles, shallow and deep occlusal steps were studied in various combinations. Buccal-lingual sections of 5 different Class 2 cavity forms, each with shallow and deep pulpal walls, were also studied. Models of the restorations placed in the various cavity forms were reproduced in a photosensitive resin (catalin). Each catalin model was placed in a matrix, mounted on the loading frame of the polariscope, and tested with a load of 168.3 pounds. The results indicate that: (1) rounding and sloping the axial wall did not consistently reduce stress in the restoration; (2) the point of load application has a definite bearing on the stress patterns induced in the restoration; (3) the cavity preparation with the rounded pulpal wall and rounded line angles showed the lowest fringe order around the boundary of the model as well as the best conformity to the boundary; and (4) extreme bulk of filling material is not always advantageous unless the radius of the circle involved (width of cavity preparation at base) is sufficiently large to permit good conformity of the stress lines to the margin of the cavity form.

76. The Effects of Cavity Preparations and Zinc Oxide and Eugenol Upon the Human Pulp. *Verda Elizabeth James, D.D.S., and Isaac Schour, D.D.S., Ph.D., College of Dentistry, University of Illinois, Chicago, Ill.* Cavities were prepared in 47 premolars of orthodontic patients and filled with zinc oxide and eugenol. The teeth were extracted 22 minutes to 39 days later. Eight additional, unoperated teeth from the same group of patients were obtained for controls. Specimens sacrificed within the 1st hour showed a disruption of the pulpodentinal membrane, a reduction of the number of odontoblasts, and edema. The edema was prominent and appeared to have pushed the odontoblasts into clumps. Some of the odontoblastic nuclei were seen within the tubules of the predentin. The layer of Weil was invaded by fibroblasts and many capillaries and also contained cellular debris. In specimens sacrificed after 2 weeks, the odontoblastic layer began to assume a normal appearance. The layer of Weil showed a reduction in the number of capil-

laries and cells. Recovery of the odontoblastic and Weil layers appeared to be complete in the later survival period (up to 39 days). These findings are in contrast to the pulpal reaction to gutta-percha fillings as reported previously. Under gutta-percha there was found in the longer survivals a persistent chronic inflammation and formation of reparative dentin. Under cavities filled with zinc oxide and eugenol, the pulp showed only minimal or occasional inflammation. There was concurrently an absence of reparative dentin.

77. Effect of Two Cementing Materials on the Gingival Tissues. *James R. Jensen, D.D.S., M.S.D., and Helmut A. Zander, M.S., D.D.S., School of Dentistry, University of Minnesota, Minneapolis, Minn.* The object of the investigation was to compare the effect on the gingival tissues of: (a) resin cement, and (b) zinc phosphate cement. Two dogs and 1 monkey were used. There were 7 gold inlays, 10 acrylic inlays, and 14 cement fillings placed with each material. Sixty days following the insertion of the fillings the animals were sacrificed and the material prepared for microscopic examination. The histologic sections showed a definite tissue reaction in the zinc phosphate cement specimens. This reaction was absent when the resin cement was used.

78. The Relationship Between Gingival Debris Accumulation, Periodontal Disease, and Dental Caries in the Syrian Hamster. *Erling Johansen, D.M.D., School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* This investigation is one of a series of experiments carried out to study the oral environmental conditions in hamsters. A total of 32 males and 23 females, 25 days of age, were littermated into 2 groups. Group I (16 males and 10 females) received caries-producing diet (*J. D. Res.* **32**: 654, 1954) and Group II (16 males and 13 females) received a similar diet without refined starch and sucrose. All animals were maintained on the experimental regimen for 230 days and examined alive at periodic intervals under ×24 to 36 magnification. The final examination was performed after sacrifice. In each successive examination the location, distribution, and character of gingival deposits were recorded for correlation with the periodontal disease picture and the occurrence of smooth and root surface caries. The results show the following differences which by statistical evaluation were significant at the 5% level: Group II has more debris, more periodontal lesions, and less caries than the littermates of Group I. On both diets there is more debris on mandibular than on maxillary molars, more debris on lingual than on buccal areas, and more caries on surfaces with debris than without debris. No sex difference in debris accumulation was found. The number of periodontal disease areas with smooth and root surface caries is significantly higher in Group I than in Group II. Group I animals have carious lesions accompanying 52% of the buccal and 81% of the lingual periodontal lesions, whereas the corresponding figures in Group II animals are 2% and 16%, respectively.

79. Types of Lactobacilli From the Oral Cavity of the White Rat. *Harold V. Jordan, M.S., and Robert J. Fitzgerald, Ph.D., National Institute of Dental Research, Bethesda, Md.* A total of 178 strains of oral lactobacilli was isolated from rats on various dietary regimens. The heterofermentative types (73 strains) were variants of *Lactobacillus fermenti*. The optimum temperature range for growth of these strains was 37 to 45° C. They could not be considered strong pentose fermenters although some variants fermented arabinose. Hippurate was not hydrolyzed and folic acid was not essential for

growth. Of particular interest was a variant of *L. fermenti* which formed golden-yellow to rust-colored pigment. Nutritional studies revealed that chromogenesis depended upon the presence of pantothenate in the medium although this vitamin was not essential for growth. Of the 105 strains of homofermentative lactobacilli, 28 strains were variants of *L. acidophilus*. Typically, they produced inactive lactic acid, fermented trehalose, but not mannitol, melibiose, or raffinose, and they did not hydrolyze hippurate. There were 2 major homofermentative groups with characteristics unlike those of any recognized species of lactobacilli. One group (24 strains) was characterized by the production of D-lactic acid, fermentation of melibiose, and raffinose but not trehalose. These strains did not hydrolyze hippurate. Certain variants fermented mannitol. The second major homofermentative group (37 strains) produced inactive lactic acid, and fermented salicin and melibiose. Melezitose mannitol, and α methyl-glucoside were not fermented. Variants of this group differed from each other in their fermentation of mannose, trehalose, and lactose, and in the hydrolysis of hippurate. The characteristics of 16 homofermentative strains were sufficiently heterogeneous to preclude assignment to a previously designated group or the establishment of a new group.

80. The Effect of Pressure on Human Dental Pulp. *Viktors Kalnins, M.D., D.D.D., D.M.Sc., L.D.S., College of Dentistry, University of California, San Francisco, Calif.* The effect obtained by pressure of a thick paste of calcium hydroxide, sulfathiazole, and strontium salts on exposed pulps was studied clinically and histologically in 262 sound and carious, permanent and deciduous teeth. Cavities exposing the pulp were prepared. After disinfection with 10% iodine solution, the opening of the pulp chamber roof was sufficiently enlarged to permit the elimination of all dentin chips during pulpal bleeding, and the wound was cleaned with 3% H_2O_2. Paste was pressed onto the pulp with a cotton pellet. Subsequently the cavities were sealed with amalgam or other filling materials. Clinical observations showed 79% of treated cases asymptomatic; a minority gave only slight discomfort of short duration. Out of 127 permanent teeth, 107 gave a vitality response after 2 months to 3 years. Fifty teeth showed a roentgenographically demonstrable "dentin bridge." Of 57 deciduous teeth, only 6 required root canal therapy or extraction after 1 month to 1⅓ years. The histologic picture obtained from 88 teeth was: (1) compression of the connective tissue stroma within the superficial part of the pulp, suggestive of the formation of a capsule or fibrous metaplasia; (2) beyond this zone, the tissue appeared undisturbed and later "dentin bridge" developed.

81. Electron Microscopy of Sectioned Connective Tissue Fibrils. *James J. Kennedy, D.D.S., M.S.D., National Institute of Dental Research, Bethesda, Md.* The fibrous components of connective tissue have been extensively examined in stained sections under the optical microscope. Considerable work has also been done with the electron microscope. The latter studies, however, have been almost entirely concerned with either dispersed or reconstituted collagen fibrils. In the present investigation, sectioned connective tissue fibrils have been examined. Small cubes of various tissues have been fixed in formalin, dehydrated in cellosolve, embedded in methacrylate, sectioned, mounted on formvar substrates, and shadowed with palladium. The most significant observation to date concerns the distinctly tubular structure of collagen fibrils in the periodontal membrane. While complete separation of the fibrils from the amorphous ground substance has been impossible, specimens have been

sufficiently purified to permit observation of striations on the internal wall of the longitudinally sectioned fibrils. A similar morphology has been observed in fibrils from other connective tissue derivatives such as cartilage, bone, subcutaneous tissue, and tendon. Close examination of the enamel matrix indicates that the component fibrils, like those in connective tissue, may also be tubular in nature.

82. The Effects of Dietary Calcium Restriction and of Phosphate Administration on the Response of Parathyroidectomized Rats to Parathyroid Extract. *Alexander D. Kenny, Ph.D., and Paul L. Munson, Ph.D., Biological Research Laboratories, Harvard School of Dental Medicine, and Department of Pharmacology, Harvard Medical School, Boston, Mass.* It was postulated that the amount of readily mobilizable calcium in rats could be progressively decreased by restricting the dietary calcium intake for increasing periods of time. Support for this idea has been provided by an experiment in which the serum calcium was determined at a specific time interval (6 hours) after parathyroidectomy, with and without administration of calcium-mobilizing hormone in the form of parathyroid extract, in rats which had been maintained on a low-calcium diet for 0, 2, or 4 days. The corresponding serum calcium values found in untreated parathyroidectomized rats were 7.3, 5.2, and 4.8 mg.%, respectively. In parathyroidectomized rats given parathyroid extract (20 U.S.P. units per 100 Gm.), the serum calcium values were 9.9, 9.0, and 8.6 mg.%, respectively. It was concluded that by various mechanisms the amount of mobilizable calcium had been decreased in proportion to the duration of restriction of dietary calcium. The rapidity with which the low-calcium diet produced its effect was especially noteworthy. The injection of a small amount of inorganic phosphate (4 mg. P per 100 Gm.) into rats depleted of calcium for 4 days before parathyroidectomy markedly decreased the response of the serum calcium level to parathyroid extract (20 U.S.P. units per gram). The mean serum calcium value was only 6.3 mg.% in the rats treated with both parathyroid extract and phosphate as compared with 8.8 mg.% in rats treated with parathyroid extract alone. The mechanism of this effect is not known.

83. A Method for Evaluation of Alveolar Bone Resorption in the Syrian Hamster. *Paul H. Keyes, D.D.S., M.S., and Harold S. Gold, A.B., Harvard School of Dental Medicine, Boston, Mass.* Measurements of alveolar crest resorption have been made in hamsters by using a polished blade of a plastic instrument graduated in 0.2 mm. units. Beneath each palatal maxillary cusp and lingual mandibular cusp measurements were made between the cervical line and the crest of the alveolar bone. Seven readings per quadrant were thus made with each value expressed to the nearest whole unit on the instrument scale. Five separate series of readings were made of 588 loci in 21 animals. There was good general reproducibility of measurement with 1,375 identical values in a total of 1,764. Scores obtained by summation of the readings confirmed the clinical impression that resorption occurs in a bilaterally symmetrical pattern. Maxillary and mandibular scores showed good correlation with scores slightly higher in the mandible. Bilateral readings of the maxillae, preferably in duplicate, appear to be adequate for evaluating bone resorption in groups of animals. A difference of 7 to 8 points in total score represents a clearly discernible difference in degree of resorption between two animals.

84. Experimental Production of Periodontal Disease in Hamsters. *G. C. Kimball, Ph.D., and T. J. Hill, D.D.S., School of Dentistry and Institute of Pathology, Western Reserve University, Cleveland, Ohio.* In a continuing study of local factors involved in the production of periodontal disease, about 30 adult male hamsters were used. Periodically, various agents were injected into the gingival sulcus around the first and second molars. These included histamine, carborundum, and cultures of *Streptococcus viridans* and yeast. Controls included untreated jaws, the injection of saline solution, or simple needle trauma. Most of the animals were killed after a few hours but 1 was treated for as long as 7 weeks. The result showed more pocket formation, bone resorption, and metachromasia of the loose connective tissue in the treated animals than in the controls. The differences were not clear-cut, probably because of the difficulty of getting sections at the exact point of treatment. To apply more effective trauma, wires were placed around the crowns of the first molars beneath the contact points. After 5 days there was some thickening of the periodontal membrane of the second molars. After 1, 2, 3, and 4 weeks, there was definite pocket formation and usually rather definite resorption of the bone and roots. Three animals were treated by histamine injection into the gingival sulcus, in addition to the wires. Two were killed after 3 weeks, and 1 after 6 weeks. In every case the first molar was either extracted or loosened and the roots broken. The 6-week animal showed pocket formation to the apex of the broken root.

85. Fluorine Uptake in Human Enamel In Situ. *Benedict B. Kimmelman, D.D.S., Albert Einstein Medical Center, N. Div., and William C. Foster, M.S., Ph.B., Hahnemann Medical College, Philadelphia, Pa.* Studies were made of uptake of fluorine by enamel of intact vital teeth in situ from a single topical application of 2% aqueous NaF, 1% aqueous Na_2SiF_6, 4% aqueous NaF, and Topi-Fluor, a commercial 2% NaF cream containing glycerine, sodium lauryl sulfoacetate, titanium diozide, and thymol. Twelve first bicuspids requiring removal for orthodontic therapy, in 3 patients aged 11 to 13, were paired as right and left homologues in each mouth. Five bicuspids received 1 application; their 5 homologues served as controls; the 6th pair remained untreated. A pair of lower second incisors requiring extraction for prosthetic reasons, in a 43-year-old woman, supplied an additional treated and control subject. The fluoride preparation was applied to the cleaned, dried, isolated experimental bicuspid for 10 to 12 minutes, the aqueous preparations being renewed during this time, and control bicuspids were coated with petroleum jelly for protection. The adult incisor received 1 5-minute application of Topi-Fluor. Extractions were performed from 4 to 51 days after the treatment, control and experimental teeth at the same time. Eleven first bicuspids in another 5 orthodontic patients, aged 11 to 18, supplied 22 control and experimental buccal and lingual surfaces; each experimental surface received a single 7-minute fluoride application and these teeth were extracted from 1 to 4 days following treatment. All patients were white. Thirty-six individual analyses of fluorine content of enamel of the 14 whole teeth and 22 treated or control surfaces, were performed by a modification of the method of McClendon and Foster (*Indust. & Engin. Chem.* [Analytical Ed.] **13**: 280-283, 1941). Analyses revealed variations of from 8 to 16% in fluorine content of whole enamel in homologous untreated teeth and untreated halves of the same tooth. No increase in per cent of fluorine content was demonstrable in the 1 application of 4% NaF. Enamel of all young teeth and surfaces receiving a single application of either 2% aqueous NaF, Topi-Fluor, or 1% aqueous Na_2SiF_6 had from 33 to 200% higher fluorine content than

controls. The treated adult tooth had 11% higher content. The 3 latter preparations showed similar results; the individual mouth, the time teeth were retained, and whether surface or tooth was treated was not significant.

86. Methods for Screening Possible Inhibitors of Dental Caries. *W. J. King, Ph.D., R. D. Manahan, B.S., and K. L. Russell, Ph.D., Colgate-Palmolive-Peet Co., Jersey City, N. J.* One approach to finding effective anticaries agents is to seek acid inhibitors which are retained in the mouth, particularly by dental plaque, and exercise a continuing restraint on acid production. Since plaque is composed, at least in part, of precipitated salivary protein, tests have been developed to determine the retention of acid inhibitors by protein matter. Precipitated salivary mucin and a commercial casein have been found to retain certain inhibitors and when these treated proteins are introduced into an appropriate nutrient with acidogenic salivary organisms, acid formation is reduced in some cases. Materials which show this effect are suggested as worthy of additional study unless their use in the oral cavity is contraindicated because of other factors, e.g., toxicity, instability, etc. Screening results by protein retention tests are presented for several dozen compounds. Among the inhibitors shown to be most effective by these tests are penicillin, Aureomycin hydrochloride, Chloromycetin, sodium-N lauroyl sarcosinate, Terramycin hydrochloride, and 5-nitro-2-furoic acid.

87. The Effect of Powdered Whole Milk–Sorbitol Diets on Dental Caries in Desalivated Hamsters. *C. E. Klapper, Ph.D., and J. F. Volker, D.D.S., Ph.D., University of Alabama, School of Dentistry, Birmingham, Alabama.* It has been observed previously in these laboratories that when desalivated hamsters are maintained on a diet made up of 30% whole wheat, 30% sorbitol, 36% whole milk powder, 3% alfalfa, and 1% sodium chloride, moderate dental caries will develop in the molars. These observations suggested that sorbitol may be considered cariogenic when the oral environment is modified. To explore this possibility further, 33 hamsters were divided into 4 similar groups. All the animals were desalivated by the removal of the submaxillary and sublingual glands and the ligation and sectioning of the parotid ducts at 27 days of age. Five days later they were placed on the experimental diets. Group 1 received 96% powdered whole milk, 3% alfalfa, and 1% sodium chloride. Group 2 received a similar diet except that 20% of the powdered whole milk was replaced by sorbitol. For Group 3 the sorbitol was increased to 40%. Group 4 was maintained on a diet of reconstituted milk made from the powdered whole milk. The animals were sacrificed 29 days later and the extent of the dental caries was recorded. The average caries score for the respective groups was determined to be as follows: Group 1 (96% powdered whole milk, no sorbitol), 26.0; Group 2 (76% powdered whole milk, 20% sorbitol), 18.8; Group 3 (56% powdered whole milk, 40% sorbitol), 14.0; Group 4 (reconstituted milk, no sorbitol), 0.5.

These results have led us to conclude tentatively that when the diet is in a powdered form some constituent of the whole milk, probably lactose, is primarily responsible for the caries observed in the desalivated hamsters. Sorbitol seems to have little or no cariogenic potential. When the milk is in fluid form it remains in the oral cavity for so short a time that no cariogenic activity is carried out, even in desalivated animals where the oral environment has proved to be especially adapted for the development of dental caries.

88. Placental Passage of Toxins. *H. T. Knighton, D.D.S., S. J. Kreshover, M.D., D.D.S., Ph.D., and J. A. Hancock, M.S.P.H., Departments of Dental*

Research and Microbiology, Medical College of Virginia, Richmond, Va. Although attention has been given to maternal influences on fetal development related to congenital disturbances of dental and other tissues, relatively little consideration has been given to the placenta's role as a barrier to injurious agents. In order to explore the role of toxins in general, tetanus was selected for initial study. Ten rabbits in 2nd or 3rd trimesters of gestation were inoculated intravenously with 50,000 to 150,000 mouse M.L.D. of tetanus toxin. Fetuses were extracted aseptically, avoiding exposure to maternal blood, at intervals varying from 15 minutes to 6 hours following injection of toxin. Either tissue extracts or blood samples were obtained from fetuses, depending upon developmental stages. Immediately after extracting each fetus, maternal blood samples were also obtained. From 0.005 to 0.2 ml. amounts of all samples were inoculated intramuscularly into hindlegs of mice. Over 100 mice injected with fetal samples showed uniformly negative results for symptoms of tetanus. Mice injected with maternal blood samples demonstrated the presence of 10 to 100 M.L.D. toxin per milliliter of maternal blood through the 1st hour and 5 to 10 M.L.D. toxin per milliliter through the 2nd hour. Preliminary evidence suggests that: (A) tetanus toxin does not pass the placental barrier, and (B) the technic used in these tests is adequate to prevent operative contamination of fetuses with maternal blood toxin. Experiments with other animals and toxins are in progress.

89. Some Responses of Lactobacilli to Thiamine. *Stewart A. Koser, Ph.D., Eleanor A. Hodges, A.B., and Joan L. Thomas, B.S., Zoller Dental Clinic and Department of Microbiology, University of Chicago, Chicago, Ill.* Results are presented under 2 headings: (1) growth response of heterofermentative lactobacilli to parts of the thiamine molecule, and (2) possible inhibitive effect of large amounts of thiamine. Under (1) it was found that a part of the thiamine molecule often may be substituted for thiamine in satisfying the needs of heterofermentative lactobacilli. The thiazole moiety of thiamine supports growth of most strains; the pyrimidine moiety does not. Thiazole and pyrimidine together, but as separate compounds, usually produce a speedier growth response than thiazole alone, although often growth is not as rapid as when thiamine itself is supplied. Evidently most lactobacilli are able to synthesize the pyrimidine moiety of thiamine, but not rapidly enough for the usual rate of growth. They are unable to synthesize the thiazole moiety of thiamine. (2) Increased amounts of thiamine up to 500 μg per milliliter caused little or no retardation of growth of most lactobacilli in a basal medium of casein digest, glucose, salts, purines, pyrimidines, and needed vitamins. Of 30 strains tested, including heterofermentative and homofermentative types of oral origin, only 1 was markedly inhibited by 100 μg and 500 μg per milliliter. Growth of most of the others was not retarded. Evidently lactobacilli are less affected by large amounts of thiamine than certain other microorganisms.

90. Peroxide as a Factor in Salivary Ecology. *Frederick W. Kraus, M.D., D.M.D., Anne P. Walker, and Kiki F. Cook, University of Alabama School of Dentistry and Veterans Administration Hospital, Birmingham, Ala.* The importance of peroxide production in saliva was assessed by estimating the numbers of those salivary bacteria which produced peroxide and those which did not. Their differentiation was accomplished by the addition of erythrocyte solution and benzidine to BHI agar. The true mean percentage of black (peroxide-producing) colonies was 58.2 ± 3.25 as calculated from an average of 21 estimations of saliva from each of 8 individuals. The individual mean percentages were highly characteristic for the individual saliva and were independent of the

mean total colony counts. The often observed inhibition of sensitive microorganisms by bacterial peroxide was inoperative in an artificial medium containing peroxidase. This respiratory enzyme was detected in saliva.

91. Prenatal Influences on the Development of Teeth. *S. J. Kreshover, D.D.S., M.D., Ph.D., O. W. Clough, D.D.S., M.S., and D. M. Bear, D.D.S., School of Dentistry, Medical College of Virginia, Richmond, Va.* To evaluate more adequately the question of maternal influences on fetal development with particular reference to dental tissues, a selected series of 30 human cases incorporating complete prenatal and pediatric histories and serially sectioned histologic material from autopsy, has been studied. Ages of delivered infants at time of death ranged from premature stillbirths to 2½ months. Of the 30 cases, 23 showed abnormal amelogenesis or dentinogenesis and 7 were negative. Changes varied from early cystic degeneration of ameloblasts and odontoblasts with temporary arrest of odontogenesis and formation of hyperplastic masses of organic enamel, to complete cessation of enamel formation. On the basis of evidence provided by length of gestation, patterns of enamel and dentin apposition, and micrometer measurements, it is concluded that 10 of the abnormal cases are prenatal in occurrence, 5 postnatal, and 8 neonatal. There is difficulty in determining whether neonatal-occurring abnormalities are true pathologic changes or manifestations of neonatal line formation in the physiologic sense. Inasmuch as the course of pregnancy was complicated in many of the cases by such disturbances as eclampsia, Rh incompatibilities, and syphilis, and that the pediatric and autopsy findings also showed wide variations of disease entities, a general correlation could be made with the manifested dental abnormalities which, however, were nonspecific in nature. It is significant that so large a percentage of cases showed prenatal disturbances of odontogenesis. It is further significant that microscopically manifested abnormalities are far more common in deciduous teeth than heretofore suspected.

92. In Vitro Effects of Antibiotics on Oral Strains of the Genus Veillonella. *Mary D. Lackey, A.B., and Robert J. Fitzgerald, Ph.D., National Institute of Dental Research, Bethesda, Md.* Six strains of Veillonella, anaerobic cocci isolated from the human oral cavity, have been subjected to antibiotics tested and the 48-hour inhibitory end point ranges were as follows: penicillin 0.4-3.1 units per milliliter, bacitracin 12.5-25.0 units per milliliter, tyrothricin 1.6-6.3 μg per milliliter, carbomycin 1.25-10.0 μg per milliliter, erythromycin 1.25-5.0 μg per milliliter, tetracycline 0.2-0.4 μg per milliliter, chlortetracycline 0.4-0.8 μg per milliliter, oxytetracycline 0.4-0.8 μg per milliliter, streptomycin 25.0-50.0 μg per milliliter, and polymyxin B 0.06-0.25 μg per milliliter. On the basis of these results it is concluded that the antibiotic sensitivity pattern of the veillonellae is intermediate between that usually considered characterisic of gram-positive bacteria and that usually shown by gram-negative bacteria.

93. Electrometric Determination of the Rate of Disaggregation and Resynthesis of Connective Tissue Following Hyaluronidase Injection. *Daniel M. Laskin, D.D.S., M.S., Norman R. Joseph, Ph.D., Richard Corley, D.D.S., and William Rominger, D.D.S., Colleges of Dentistry and Pharmacy, University of Illinois, Chicago, Ill.* The state of aggregation of the negatively charged colloids of the connective tissue can be estimated using an electrometric method (Joseph, Engel, and Catchpole, *Biochem. et biophys. acta* **8**: 575, 1952). A potential difference, E_d, occurring at a boundary between con-

nective tissue and a $\frac{1}{10}$ dilution of isotonic saline is measured. The colloid charge density (X) is related to this dilution potential by the approximation formula $E_d = -12.3 + 215$ X. The method has been applied to determine the rate and degree of disaggregation and resynthesis of some connective tissue components of rabbit skin following the injection of hyaluronidase. The abdominal wall was used as the test site with the subcutaneous tissue of the hindleg serving as a reference point. Base-line potentials were established with 0.15 M NaCl at both junctions. Dilution potentials were measured by substituting 0.015 NaCl at the abdominal junction. The average potential difference (E_d) was + 3.1 m^v corresponding to 0.071 equivalent of negatively charged colloid. Following the injection of 5-7 turbidity reducing units of hyaluronidase (Wydase) there was a drop of about 10 m^v during the first 10 minutes. The lowest E_d was obtained after 30 minutes at which time the mean value was –8.8 m^v (0.016 equivalent of negatively charged colloid). After 1 hour the values tended to return toward normal with complete recovery usually occurring within 8 hours after the time of injection. The electrometric readings are sensitive measures of the influence of hyaluronidase on connective tissue ground substance.

94. The Effect of Embryo Extract on the In Vitro Culture of Tooth Germs. *William Lefkowitz, D.D.S., and Patricia Swayne, A.B., School of Dentistry, Ohio State University, Columbus, Ohio.* Organized growth requires nutritional elements which will provide growth and development in addition to cell proliferation. Various concentrations of rat embryo extract produced changes in the development of the enamel epithelium and papilla. When cultivated in the clot with dilute embryo extract, harmonious development of the components of the tooth germ occurred as measured by in vivo development. When planted on the clot, which exposed the explant directly to dilute embryo extract, development of the papilla was retarded and the enamel epithelium advanced. Concentrated embryo extract applied directly to the tooth germ provided the necessary elements for the papilla and inhibited development of the enamel organ. In consideration of the results of cultivation in an embryo extract-free medium, filtering the extract through the coagulum provides a more desirable medium.

95. Further Studies on the Effect of Lactones on Synthetic Calculus Formation. *S. Wah Leung, D.D.S., Ph.D., School of Dentistry, University of Pittsburgh, Pittsburgh, Pa.* Certain sugar lactones have been shown to inhibit synthetic calculus formation on extracted teeth when added to a final concentration of 0.5% to the saliva in which the teeth are suspended. The question arises as to whether they would be effective if permitted to act on the teeth for only a few minutes each day, simulating their clinical use as a mouthwash or dentifrice. Instead of adding the lactone to the saliva, therefore, they were made up in a phosphate buffer at pH 7.0 ± 0.2. At 9 A.M. and 11 P.M. daily, the teeth were dipped into the solutions and mildly agitated. Control teeth were dipped into phosphate buffer without lactone. In between dippings the teeth were suspended in saliva in the cyclic immersion apparatus previously described. In the first such experiment galactono- and glucuronolactones were used as 0.5% solutions in M/15 phosphate buffer. After 3 weeks no differences were detected in the amounts of calculus collected from the 3 groups of teeth. The experiment was repeated with 5.0% lactone solutions in phosphate buffer. After only 2 weeks the lactone-treated teeth showed markedly less calculus. From 10 control teeth 17 mg. of material was collected

while only 7 mg. and 6 mg., respectively, were collected from the 10 teeth in each of the 2 lactone solutions. Thus, treating the teeth with 5.0% lactone for two 2-minute periods each day reduced the deposition of calculus.

96. Tumors of the Notochord of the Salamander, Amblystoma Punctatum, Produced by Crystalline Lathyrus Factor. *Barnet M. Levy, D.D.S., M.S., and Gabriel Godman, M.D., Department of Dental and Oral Surgery and the Histochemical Research Laboratory, Faculty of Medicine, Columbia University, New York, N. Y.* *Amblystoma punctatum* embryos raised in a 1 mg.% aqueous solution of crystalline *L. odorata* showed foreshortening of the mandible and forelimbs. Tumors of the notochord also developed. The histologic appearance of these tumors is described. Two possible mechanisms of pathogenesis of these lesions are discussed. The suggestion is made that these tumors arise as a consequence either of a focal degeneration of the notochord sheath leading to secondary ecchordoses or prolapses, or of a direct stimulation to proliferation of the notochordal cells.

97. The Effect of Fluorine on the Deposition of Calcium in the Rat Tibia. *R. C. Likins, D.D.S., R. O. Scow, M.D., M.S., I. Zipkin, Ph.D., and A. C. Steere, B.S., National Institute of Dental Research and National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.* Pairmates from 12 litters of female Sprague-Dawley rats weighing 30 to 40 grams were assigned to 2 groups. A metal pin was surgically inserted in the left tibia of each rat. The first group received 0.33 mg. of fluoride (NaF) daily by stomach tube for 30 days; the 2nd was intubated with distilled water. All received 0.66 μc of $Ca^{45}Cl_2$ by intraperitoneal injection following each intubation. The lengths of the diaphyseal segments proximal and distal to the pin were measured on radiographs taken at the beginning and end of the experiment, and the growth increments obtained by difference. The tibiae were excised and the proximal epiphysis (PE) removed by sectioning through the epiphyseal plate. The original diaphyseal segment (ODS) was then severed from the proximal diaphyseal increment (PDI) at one end, and the distal diaphyseal increment plus distal epiphysis (DDI + DE) at the other. The segments were ashed and analyzed for fluorine and radiocalcium. Results: (A) the fluorine contents (per cent) were dissimilar ($p < 0.01$) in all segments, and in the order PE > DDI + De > PDI > ODS; (B) the original diaphyseal segment contained significantly less ($p < 0.01$) radiocalcium (per cent) than the other segments, which did not differ ($p > 0.05$) from one another; (C) fluorine had no effect ($p > 0.05$) on the growth or the deposition of Ca^{45} in the various segments. The data indicate that the deposition of fluorine was not proportional to the uptake of calcium.

98. Wound Healing in the Jaws of Hamsters With Alizarin Red S Vital Dye. *Robert H. Linn, D.D.S., M.S.D., School of Dentistry, University of Minnesota, Minneapolis, Minn.* Lower first molars were extracted from 40 Syrian hamsters, and injections of alizarin red S were given. The animals were sacrificed at intervals of from 12 hours to 30 days after extraction. The healing process was studied by comparing paraffin sections stained with hematoxylin and eosin, ground-sections stained with alizarin red S, and radiographs at the various stages of healing. The over-all healing process following uncomplicated extraction in the hamster had the same fundamental pattern reported in other experimental animals, namely: (1) formation of blood clot, (2) organization of clot, (3) deposition of new bone, (4) calcification of new bone, (5) physiologic reconstruction of the extraction area. The first appearance of new bone was at

7 days in the fundus and lateral walls of the alveolus. The calcification of the new bone began between 7 and 10 days after extraction in the fundus of the alveolus, and was completed at about 15 days as indicated by the deposition of alizarin red S. From 15 to 30 days the principal change was that of functional reconstruction. The radiographic appearance of the sockets paralleled that of the ground sections at the various stages of healing. Retention of foreign bodies such as wood shavings and necrotic bone in the sockets appeared to delay the healing process. No discernible changes in the vital organs resulted from alizarin red S in the dosages used.

99. Whole Saliva Enzymic Activities and Characteristic Patterns Related to Oral Conditions. *V. F. Lisanti, D.M.D., and H. H. Chauncey, M.A., Tufts College Dental School, Boston, Mass.* There appears to be a significant relationship between certain whole saliva hydrolytic enzymes and oral disease processes. Of 10 enzymes previously studied, the following were found to be of diagnostic value: hyaluronidase, cholinesterase, β-glucuronidase, and lipase. A direct correlation was obtained with each of these 4 enzymes and the number of carious and carious plus restored teeth. Characteristic enzyme patterns have been found in a mixed population of male subjects in which the oral condition was categorized into the following classifications: "normal," dental caries, periodontal disease, dental caries plus periodontal disease, and edentulous. These patterns permitted identification of the individual clinical entities. It was possible to select the caries group in 75 to 80 per cent of the cases, the periodontal disease group 80 to 99 per cent, and the mixed condition 60 to 70 per cent. These enzymes have been found to be elaborated by oral microorganism isolates obtained from saliva, carious lesions, and periodontal pockets.

100. Distribution of Inorganic Carbonate in Human Enamel. *Marguerite F. Little, B.S., and Finn Brudevold, D.D.S., M.S., Eastman Dental Dispensary, Rochester, N. Y.* Successive layers of enamel were ground from unerupted and erupted teeth of different ages, and after drying at 110° C. to constant weight the concentrations of CO_2 were determined using a Van Slyke-Folch manometric apparatus. CO_2 was present in all samples, but the concentration was lowest in the outer layer of the enamel and increased to 2.3 to 2.4 per cent in the subsurface enamel. The CO_2 content of the superficial enamel was higher in unerupted teeth (1.6 to 1.7 per cent) than in erupted teeth (1.3 to 1.4 per cent).

101. The Pathogenic Components of an Experimental "Fusospirochetal" Infection. *J. B. MacDonald, D.D.S., Ph.D., R. M. Sutton, M. L. Knoll, B.S., and E. M. Madlener, D.D.S., Faculty of Dentistry, University of Toronto, Toronto, Canada.* Experimental "fusospirochetal" infection has been reproduced in guinea pigs by recombining pure cultures of 16 distinct strains of bacteria isolated from a guinea pig fusospirochetal exudate. Analysis to determine the essential components has been carried out by systematically deleting organisms from recombination mixtures. The mixtures grown on wheel plates (previously described) were emulsified and inoculated into 4 or more guinea pigs and transmitted serially through 4 or more passages wherever possible. Results were assessed statistically in terms of infectivity of the mixtures (1st passage) and transmissibility of the infection (subsequent passages). A combination of not less than 4 organisms appeared to be essential to produce an infection comparable to the original infection produced with all 16 strains. The 4 strains included 2 strains of Bacteroides, 1 motile gram-negative rod,

and an aerobic diphtheroid. Spirochetes, fusiforms, vibrios, and anaerobic streptococci were found to be noncontributory. Based on these findings an experiment was undertaken to compare directly the results of inoculating the minimum combination of 4, the remaining 12, and the total group of 16 strains. Typical and indistinguishable infection was produced by combinations of 4 or 16. The combination of 12 failed to produce infection.

102. Studies on Spirillum Sputigenum. *J. B. MacDonald, D.D.S., Ph.D., and E. M. Madlener, D.D.S., Division of Dental Research, Faculty of Dentistry, University of Toronto, Toronto, Canada.* Flagellation emanating from the concave surface of curved bacteria has been described for organisms of 3 different habitats: (1) the oral cavity of human beings *(Spirillum sputigenum)*, (2) guinea-pig caecum *(Selenomonas palpitans)*, (3) rumen fluid of herbivores *(Selenomonastix)*. Such flagellation is unique and has prompted a proposal that a new genus, Selenomonas, be recognized. Of the 3 apparently related or identical species only the oral organisms have been studied in pure culture. Eight strains studied in pure culture appear to be comparable in morphologic, cultural, and biochemical characteristics, and in respect to pathogenicity in experimental animals. Dark-field examination, flagellar stains, and electron photomicrography suggest that the flagellation may be randomly distributed about the circumference and that flagellation from the concave surface is only 1 of several points of origin of the flagella. If further investigation should establish this point it would invalidate the basis for recognizing the genus Selenomonas.

103. Lipolytic and Esterolytic Activity of Salivary Organisms. *I. R. Mahler, M.A., H. H. Chauncey, M.A., and V. F. Lisanti, D.M.D., Tufts College Dental School, Boston, Mass.* The ability of whole saliva to hydrolyze aromatic esters of acetic and lauric acid has been demonstrated. Since parotid saliva was observed to contain only a small fraction of the activity of whole saliva, the presence of these enzymes has been attributed mainly to oral microorganisms. In the present study 2 additional esters of beta-naphthol, mysterate, and stearate, were employed to differentiate between the esterolytic and lipolytic properties of saliva and salivary organisms. Whole saliva samples were centrifuged and the sediment, consisting chiefly of microorganisms, and the supernatant were tested against the 4 substrates. In all cases, both sediment and supernatant exhibited very strong activity against the acetate substrate. Decreasing activity was noticed in the following order: acetate > laurate > mysterate > stearate. Microorganisms isolated from saliva and dental plaques showed marked variance in their ability to attack these substrates. Sediments obtained from cultures of *Streptococcus salivarius* and a Lancefield Group "L" streptococcus were weak to moderate in their activity against the acetate and were unable to hydrolyze the other 3 esters. *Lactobacillus casei* was found to attack acetate, laurate, and mysterate, but seemed unable to hydrolyze stearate. One strain of *Streptococcus mitis* demonstrated the ability to attack all 4 substrates vigorously. Isolates identified as *Micrococcus pyogenes* var. *aureus* showed marked hydrolytic powers against all the esters. It was the only organism tested which showed strong enzymatic activity in the culture filtrate. This preliminary study shows that many salivary microorganisms are only active against small-chain fatty acids. Some of those which demonstrate short-chain activity also have hydrolytic potential against long-chain fatty acid esters. Further differentiation is obtained in the comparison of the exogenous and endogenous production of these enzymes.

104. Comparison Between Inhibition of Glycolysis and Bactericidal Action of Biologically Active Compounds. *I. R. Mahler, M.S., R. S. Manly, Ph.D., Tufts College Dental School, Boston, Mass.* The pH attainment of bacterial cell sediments has been studied under conditions of controlled temperature, buffer capacity, and substrate concentration. The effect of inhibitory compounds on bacterial glycolysis has been measured. Studies were carried out on dental plaque, salivary sediment, and representative strains of microorganisms isolated from dental plaque material. The compounds tested included specific enzyme inhibitors, inorganic salts of heavy metals, and germicidal agents. All results, listed in terms of inhibition recovery of acid production, were based on a 20-minute exposure to the test substance and 30-minute recovery in glucose buffer. The bactericidal effect of the inhibitors was measured concurrently with their effect on the glycolytic cycle. Of the 16 compounds tested, 7 caused marked to moderate increase in pH levels. Among the active compounds, sodium fluoride in 1 per cent concentration and iodoacetate at 0.01 per cent were the only nonbactericidal agents. The 5 remaining active substances—triethylcarbinol, 1, 3-propanediol, iodoacetamide, N(3 aminopropyl) rosin amine D diacetate, and sodium N-lauroyl sarcoside—inhibited the growth of *Streptococcus mitis, Streptococcus salivarius,* and *Lactobacillus casei. Aerobacter aerogenes* proved more resistant than the gram-positive strains. Of the 9 remaining compounds, Zephiran chloride, copper sulfate, 2(ethylhexyl 3-aminopropylether), N, N dimethyl-dodecyl amine, dodecyl tris(2 hydroxyethyl) ammonium were bactericidal while showing little or no effect against acid production.

105. The Effect of a Therapeutic Dentifrice on the Dental Needs of Children. *Anthony J. Malone, D.D.S., University of Illinois Foundation, Chicago, Ill.* A 10-year study has been started for the clinical evaluation of a therapeutic dentifrice against a placebo dentifrice by observing the trend in dental needs of children. The evaluation will be made on randomly selected white children, of both sexes, who are receiving routine and, insofar as possible, identical care and instruction from 4 pedodontists in their private practices. In each practice the study will include a maximum of 60 children, consisting of 10 boys and 10 girls, 4 years, 8 years, and 12 years of age. Age groups have been confined to single years. This restriction ensures a more homogeneous population of dentitions and thus simplifies the problem of clinical examination and interpretation. A clinical and radiographic examination of each patient is made by the pedodontist and recorded on a specially designed chart. Salivary lactobacillus counts for each patient are made before and after all carious teeth are restored. Following complete oral rehabilitation, the dentist places each patient on a toothbrushing program utilizing the therapeutic or placebo dentifrice initially selected at random. Patients are recalled every 4 months for clinical and radiographic examination. Lactobacillus counts are made periodically. The effectiveness of each dentifrice will be evaluated by comparing the dental needs of the experimental subjects with those of the controls. Early observations reveal that excavation of lesions and restoration with sedative dressings are coincident with a dramatic decrease in lactobacillus counts.

106. Social Attitudes in Relation to Caries Formation. *John H. Manhold, D.M.D., and Marshall B. Jones, Ph.D., U. S. Naval School of Aviation Medicine, Naval Air Station, Pensacola, Fla.* Empirical study by Manhold and his associates has demonstrated a consanguinity between dental decay and neurotic tendencies. Parallel to medical psychosomatic hypothesization, neurotic conditions (through physiologic mechanisms) may be contributory to dental caries. Furthermore, because of this possible parallelism, research to

date has been channelized into this conversion from neurosis theory. Recently, Jones and Manhold examined a hitherto unexplored and decidedly less spectacular psychosomatic possibility—that normal "nonneurotic" adults who are remiss about their physical health may possess identifiable psychologic traits. By use of a widely employed psychologic instrument (the California F scale), they discovered that persons with certain social attitudes are in poorer physical health generally. Dentistry has long believed that neglect of oral health aids caries formation. The present study, therefore, is an empirical examination to ascertain whether this less dramatic psychosomatic possibility is pertinent to dental pathology. The California F Scale of authoritarianism and Bodecker DMF ratings were employed as systems of measurement on a sample of 1,019 cadets. Results showed that a nonauthoritarian group of 144 subjects had a mean DMF score of 30.46. The remaining 875 subjects had a mean DMF score of 26.69. The "t"-test value calculated was 2.69; significant at the 0.01 level. Further testing of this psychosomatic possibility and of the conclusion that the nonauthoritarian personality presents more dental decay through attitudes of neglect is under way.

107. Comparison of Effects of Acid Decalcification and Chelation at a Neutral pH Upon Histochemical Staining. *Dorothy F. Mardfin, A.B., and Verda Elizabeth James, A.B., D.D.S., University of Illinois, College of Dentistry, Chicago, Ill.* Premolars of adolescent patients were decalcified in 5 per cent nitric acid and 0.5 M sodium salt of ethylenediamine tetracetic acid in phosphate buffer solution at pH 7.4. Chelation for approximately 10 days was sufficient, provided the teeth were ground to 2 mm. slabs. Roentgenograms were used to establish the completion of decalcification. Rat cerebellum, unchelated and chelated, was used as control. Ten per cent neutral formalin was used for fixation. The specimens were embedded in either paraffin or celloidin, and the sections were stained with hematoxylin and eosin, Feulgen, and thionin. There was no apparent difference between the celloidin- and paraffin-embedded material. In chelated specimens stained with Feulgen, the DNA of the cells gave a positive reaction in contrast with the sections stained with nitric acid, which showed a negative result. Chelated specimens stained with thionin showed typical positive staining of the nucleic acids in the nucleus, while those decalcified with nitric acid were negative. The teeth decalcified by chelation showed higher contrast and finer details with hematoxylin and eosin than did those that were decalcified with nitric acid. Chelation up to 56 days did not affect the staining reactions.

108. Experimental Pulpotomy in Incisors of the Rat. *Maury Massler, D.D.S., M.S., J. G. Perreault, D.D.S., M.S., and Isaac Schour, D.D.S., Ph.D., College of Dentistry, University of Illinois, Chicago, Ill.* The testing of different substances used for pulpotomy in human teeth is limited because large numbers of specimens are difficult to obtain for histologic assessment and clinical criteria are often inconclusive. The method previously reported for preparing cavities in lower incisors of the rat at the level of the mental foramen was therefore modified to produce pulpotomies under relatively well-controlled experimental conditions. Over 100 animals were operated upon and sacrificed at 3-, 7-, 10-, 14-, and 21-day intervals. Calcium hydroxide, dicalcium phosphate, or zinc oxide–eugenol paste was used to cover the amputated pulp stumps. Histologic analysis indicates the following common pattern of pulp healing and zones under each of the 3 substances tested: (1) acute inflammation with predominance of neutrophils; (2) degeneration and necrosis of the pulp immediately subjacent to the filling substance; (3) diffuse calcification of the necrotic layer; and (4) occasional bridging by dentinlike structure. The 3

substances tested produced slight differences in degree of healing, but the basic pattern of healing was similar. Further studies are being conducted to determine the role of the blood clot in pulpal healing.

109. Reaction of Fluorine in Vivo. *H. G. McCann, M.S., National Institute of Dental Research, Bethesda, Md.* Weanling rats (200) were divided so that equal numbers of each litter were placed in each of 2 groups. Both groups were fed an adequate, low-fluorine diet. One group received 100 ppm fluorine as NaF in the drinking water; the controls received distilled water. After 100 days the animals were sacrificed, and dry, fat-free samples (60 mesh powder) of incisor enamel and dentin, molar enamel and dentin, mandibles, femurs, and humeri were prepared. These were analyzed for Ca, P, Mg, F, Na, CO_2, and ash. The solubility of the samples in water, HCl, and CH_3COOH was determined by analysis of the Ca and P extracted by these solvents. The solubilities of the in vivo fluoride-treated specimens were considerably lower than those of the controls and resembled those of control samples treated in vitro with NaF solutions. Previous work has shown that fluoride treatment in vitro results in the production of fluorapatite almost exclusively. Fluorapatite, therefore, may be assumed to be one of the principal products formed in the hard tissues of fluoride-treated rats. The exchange of OH^- with the F in the samples was also determined, using NaOH of various strengths. Reactions with OH^- show that fluorine incorporated into the crystal in vivo is not readily available for exchange, whereas most of the fluorine retained by the material in vitro is surface bound and readily exchanges with OH^-.

110. Dental Caries and Rate of Growth in Three Strains of White Rats. *F. J. McClure, Ph.D., and F. L. Losee, M.S., D.D.S., National Institute of Dental Research and Naval Medical Research Institute, Bethesda, Md.* A cariogenic diet (636) containing "dry autoclaved" skim milk powder 35 per cent, corn starch 45 per cent, cerelose 18 per cent, and powdered whole liver 2 per cent was fed ad libitum to 36 Osborne-Mendel, 24 Holtzman, and 29 Sprague-Dawley weanling-aged rats from the colony at NIH; these susceptible-to-caries rats were housed at the NIH. Twenty litters (4 rats per litter) of Osborne-Mendel weanling-aged rats from the colony at the NMRI were housed, 2 rats from each litter at the NIH, and 2 at the NMRI. The rats' average daily weight gains, which were below normal because of deficient protein in the diet, were as follows: Sprague-Dawley (NIH) 0.52 grams, Holtzman (NIH) 0.30 grams, Osborne-Mendel (NIH) 1.15 grams, Osborne-Mendel (NMRI) at NIH 0.82 grams, Osborne-Mendel (NMRI) at NMRI 1.45 grams. Sprague-Dawley (NIH) and Holtzman (NIH) rats showed, respectively, 96.6 per cent and 100.0 per cent incidence of smooth surface caries, 31.8 per cent and 33.3 per cent occlusal surface caries, whereas Osborne-Mendel (NIH), Osborne-Mendel (NMRI) at NIH, and Osborne-Mendel (NMRI) at NMRI showed, respectively, 69.4 per cent, 59.1 per cent, and 68.3 per cent smooth surface caries, and 0.0 per cent, 88.6 per cent and 85.4 per cent occlusal surface caries. Caries scores in these latter 3 groups were, respectively, 2.3, 8.5, and 7.9. Caries scores averaged 9.4 in Sprague-Dawley (NIH) rats and 8.3 in Holtzman (NIH) rats. The caries data indicate pronounced differences in the relative amounts of smooth surface caries versus occlusal surface caries in the Sprague-Dawley and Holtzman rats versus Osborne-Mendel (NMRI) and the Osborne-Mendel (NIH). Environmental conditions seemed to have had an effect on rate of weight gain of Osborne-Mendel (NMRI) rats under ad libitum feeding, but apparently did not affect caries production. In these experiments rate of daily gain was not related to incidence or severity of caries.

111. Water Balance in Pantothenic Acid Deficient Rats. *Ilmar Merits, B.S., L. S. Fosdick, Ph.D., and J. C. Calandra, Ph.D., M.D., Northwestern University Dental School, Chicago, Ill.* During some studies on permeability of the membranes of the pleural cavity there were indications that acetyl groups were a controlling factor in the transport of water from the pleural cavity to the blood. This in turn suggested that coenzyme A played a role in the permeability of the tissues. Rats were made pantothenic acid-deficient and the passage of water through irritated pleural cavity membranes was measured in these rats and in normal rats. It was found that the water passed from the pleural cavity to the blood stream in the pantothenic acid deficient rats much more rapidly than in normal rats. There were indications that the adrenal cortex was affected by the pantothenic acid deficiency.

112. The Nature of the Gingival Plaque of the Hamster: Production, Prevention, and Removal. *David F. Mitchell, D.D.S., Ph.D., and Marilyn Johnson, B.S., School of Dentistry, University of Minnesota, Minneapolis, Minn.* Hamsters maintained on a high carbohydrate diet develop grossly visible white plaques at the palatal gingival line which have been shown to be associated with cervical caries and gingival recession. The number of gingival areas involved next to the lingual surfaces of the 6 upper molars may be scored in the living animal with or without magnification. Laboratory chow fed to these animals for 2 weeks removes this visible plaque. A high carbohydrate diet fed to the same animals for 2 weeks reproduces the plaque. Penicillin added to the carbohydrate diet removes the plaque in 2 weeks. The plaque was studied by microscopic examination, pH changes in vitro, selective culture and other methods and it was found to consist, in large part, of aciduric organisms. The ease of production and removal of the plaque by dietary methods may lead to a short-term screening test for potential caries-inhibiting agents. Speculation is made on the possible relationship of these findings to dental caries and periodontal disease in the human being.

113. Growth Pattern of the Rat Maxilla. *Clive I. Mohammed, L.D.S., M.D., College of Dentistry, University of Illinois, Chicago, Ill.* This study was based on complete serial histologic sections of the heads of 41 *ia* mutant rats, which are characterized by a marked retardation of bone resorption, and 40 normal littermates. The ages of the animals ranged from 16 days' insemination age to 30 days after birth. The nonresorbing bone of the *ia* rats affords permanent landmarks which may be used as guides for tracing the amount and direction of bone growth, and thus provides a unique opportunity for studying the growth pattern of various bones. Heterozygous littermates of the *ia* rats do not differ in their growth and development from normal animals of the stock albino strain. Apposition of bone was determined by superimposing camera lucida tracings of identical regions of the *ia* maxillae at successive stages. Resorption was studied by superimposing tracings of comparable regions of *ia* and normal maxillae at successive stages. It was found that the maxilla of the rat arises from 1 center of ossification at 17 days' insemination age, at which time most of its processes can be distinguished. The lateral nasal cartilage of the nasal capsule acts as a template lateral to which the maxilla molds itself. Three cartilages designated as the palatine, maxillary, and nasopalatine cartilages were found associated with the growing rat maxilla. While sutural growth plays a predominant role in the increase in length and height of the maxilla, increase in width occurs by the growth of the palatine symphysis, the junction of the palatin cartilages in the midline.

114. Mesiodistal Crown Diameters of the Deciduous and Permanent Teeth in Individuals. *Coenraad F. A. Moorrees, D.D.S., Elli Jensen, Tandlaege, Selma Thomsen, Tandlaege, and Peter Kai-jen Yen, D.D.S., D.M.D., The Forsyth Dental Infirmary for Children, Boston, Mass.* As part of a longitudinal growth study, dentition measurements were made on dental casts with a sliding caliper. The errors of measurements between different observers, expressed as the standard deviation of the differences, is 0.09 mm. (n = 360). Mesiodistal crown diameters of the teeth are larger in the males than in the females. This sex difference is more pronounced in the permanent than in the deciduous dentition. The greatest differences are observed between the canines; the critical ratio is 6.56 for the maxillary canines and 8.60 for the mandibular canines; and for the deciduous canines C. R. is 3.68 and 3.16, respectively. The mean ratio between the sum of the mesiodistal crown diameters of the deciduous teeth and that of the corresponding permanent teeth $\frac{(dm_2 - dm_2)}{(Pm_2 - Pm_2)}$ in the maxilla is 92.92 per cent for the males and 94.77 per cent for the females; in the mandible the findings are 98.28 and 100.23 per cent, respectively. The coefficients of correlation for the combined mesiodistal crown diameters of the deciduous and their corresponding permanent teeth range between 0.49 and 0.69. The correlation coefficients for single teeth range from 0.23 to 0.65.

115. A Comparison Between the Anticariogenic Effect of a Stannous Fluoride- and Sodium Fluoride-Containing Dentifrice. *Joseph C. Muhler, D.D.S., Ph.D., Harry G. Day, Ph. D., William H. Nebergall, Ph.D., and Arthur W. Radike, D.D.S., Indiana University, Bloomington, Ind., and Cincinnati, Ohio.* A clinical study on children, aged 6 to 15 years, was conducted in Bloomington, Indiana, to compare the effectiveness of a dentifrice containing stannous fluoride with an otherwise identical dentifrice containing sodium fluoride. Stoichiometrically equivalent amounts of fluorine were added to each dentifrice. The anticariogenic effect of each was compared with a control group using the same dentifrice without added fluorides. The results after the children had used the dentifrices for a period of 1 year indicate that the dentifrice containing sodium fluoride was essentially without effect in reducing the dental caries experience, while the children using the dentifrice containing stannous fluoride had a definite reduction in the incidence of dental caries. On the basis of the total DMF surfaces the children using the sodium fluoride had a reduction of 10 per cent while a 36 per cent reduction was found in the children using the stannous fluoride. The DMF teeth index indicated a 2 per cent reduction for those children using the sodium fluoride dentifrice and a 34 per cent reduction for the stannous fluoride dentifrice. These findings with the latter dentifrice are similar to those in a clinical test on adults. In this case the caries reductions were 43 per cent by DMFS and 54 per cent by DMFT. Similar agreement was found in a separate clinical test of the stannous fluoride dentifrice on children and in a 4th test where the SnF_2 was topically applied to the erupted teeth of children.

116. A Rapid and Precise Semimicro Method for the Analysis of Calcium. *Paul L. Munson, Ph.D., Oscar A. Iseri, B.S., Alexander D. Kenny, Ph.D., Victor Cohn, M.S., and Mindel C. Sheps, M.D., Harvard School of Dental Medicine, Harvard Medical School and Harvard School of Public Health, Boston, Mass.* A procedure has been developed for the quantitative estimation of calcium in blood serum and other biologic materials which combines the advantages of several previously published methods based on the titration with ethylenediamine tetraacetate (EDTA) with ammonium purpurate as indicator. The

titration is carried out by addition of standardized EDTA solution from a microburet directly into the unknown solution in a cuvette in place in a spectrophotometer. The solution is stirred after each addition of EDTA by bubbling filtered nitrogen or air intermittently through a thin glass tube which extends to the bottom of the cuvette. The galvanometer reading is recorded after addition and mixing of each increment of EDTA and the end point is determined by the intersection of the 2 straight lines resulting from a plot of the galvanometer readings. The titration can be completed in 2 minutes. The precision of the method is 0.2 mg. per cent or better, using 0.5 ml. of serum, and is satisfactory for many purposes with as little as 0.1 ml. of serum. With some limitations the method can also be applied to the quantitative analysis of bone, dentin, enamel, milk, and urine.

117. Preliminary Evaluation of Tooth Transplantation Technics in the Hamster. *Hugh I. Myers, Ph.D., and Viola D. Flanagan, B.S., School of Dentistry, University of Kansas City, Kansas City, Mo.* Previous studies have indicated that replantation of teeth in the hamster can be a successful procedure leading to periodontal membrane reattachment, and viable pulp and minimal root resorption. Since transplantation involves fitting the tooth into a socket other than its own, it would be expected that positive results would be more difficult to attain. Experiments conducted by some of the oral surgery students have demonstrated this to be true. One experiment consisted of autotransplantation of the mandibular 2nd molar to the opposite side. The sockets were prepared by reaming with a small burr. Of 12 animals, only 4 survived this procedure 30 days postoperatively. Two of these 4 did not retain the transplants. Neither of the remaining 2 showed regeneration. One tooth showed epithelium completely lining the socket and beginning to extend into the pulp. Heterotransplants of 2nd molars with curettage for socket preparation yielded negative results. Autotransplantation of mandibular 2nd molars that involved extraction, socket preparation with burr or curet, a time interval of 1 or 2 weeks to allow granulation tissue to form, and then insertion of the freshly extracted tooth also yielded negative results. Thirty-day-old hamster 3rd molar transplants without socket preparation have yielded positive results. Heterotransplants involving transfer of the 3rd molars on the same side of the mandible provides a tight fit, but allows complete insertion of the root into the socket. One of the most important factors for a successful result seems to be immobilization of the transplant.

118. Work Hardening of the Enamel. *H. H. Neumann, M.D., School of Dental and Oral Surgery, Faculty of Medicine, Columbia University, New York, N. Y.* The cyclic strain of vigorous chewing has a measurable impact on the enamel structure. The microstructure of the enamel may respond to chewing both as a physical conglomeration containing dispersed crystals, and as a biologic unit. Physical methods can be applied to examine molecular response in the enamel to the stress of chewing: the measurement of variations of x-ray diffraction, of the rate of isotope uptake, and of corrosion resistance. Further figures and curves are given for isotope uptake of molars with and without the stress of chewing: the initially increased uptake of labeled phosphorus in the nonchewing molars of monkeys is followed after periods of varying length by a relative decrease in the P_{32} uptake, compared with the chewing molars. The pulsating load of vigorous chewing, inconsequential in each single impact, produces through repetition of this cyclic strain molecular dislocations, as a measurable impact on the enamel. The sensitivity of the enamel to the stress of intermittent compression is a property common to biologic

structures as well as to minerals, ceramics, glass, and metals (work hardening). In the enamel, physical reactions, due to compression stress, are more readily discernible than biologic effects. This does not preclude, however, that biologic effects may have the more significant practical consequences of influencing immunologic and corrosion resistance, that is, caries resistance.

119. The Effect of Alloxan Diabetes on Caries Incidence in the Albino Rat. *Martin S. Nichols and James H. Shaw, B.A., Ph.D., Harvard School of Dental Medicine, Boston, Mass.* Reports on caries experience among diabetics are contradictory; in addition, interpretation of these results is limited because of varying degrees to which the diabetic process is controlled. The use of alloxan for production of a diabetic syndrome in rats provides an experimental approach to study this relationship. In Experiment 1, 69 caries-susceptible albino rats were divided at 36 to 37 days of age into 2 groups, according to sex and littermates. After 18 hours starvation, 41 were injected intravenously with 1 dose of alloxan monohydrate at 48 mg. per kilogram of body weight; 28 served as controls. All animals consumed a cariogenic diet ad libitum from weaning until sacrificed at 139 to 140 days of age, when caries was evident clinically. Monthly blood sugar determinations were made; 14 remained diabetic for the entire period with blood sugar values over 400 mg. per cent in contrast to under 200 mg. per cent for controls. In Experiment 2, 71 animals were divided into 3 groups, according to sex and littermates. Eighteen uninjected animals and 31 animals injected as in Experiment 1 were provided food ad libitum. Another 22 uninjected animals were given daily weighed quantities of food equal to that consumed by injected littermates of the same sex. The design of this experiment otherwise followed that of Experiment 1. The diabetic animals had a high incidence of cataracts, high food consumption, and body weights substantially below the controls. There was no statistically significant difference between caries scores for the diabetics and controls.

120. Some Nonprotein Nitrogen Constituents of Blood and Saliva and Their Relation to Dental Caries. *Gordon Nikiforuk, D.D.S., M. A. Cox, M.D., S. R. Jackson, Ph.D., and R. M. Grainger, D.D.S., M.S., D.D.P.H., Hospital for Sick Children, and Faculty of Dentistry, University of Toronto, Toronto, Canada.* This report deals with the interrelationships between blood urea, salivary urea, ammonia, and the effect of these constituents on dental caries. A method for analysis of microquantities of urea in 0.2 ml. of blood was developed. Subsequent to the development of suitable techniques 94 7-year-old children and 40 caries-free children have been studied with regard to the following characteristics: blood urea, salivary urea and ammonia, rate of ammonia production and urea disappearance after incubation for 2 hours, and annual caries increment. Statistical analysis of the data indicates that there is a highly significant correlation between blood urea nitrogen and salivary urea nitrogen; also, between salivary urea plus ammonia nitrogen and the urea nitrogen in the blood. There is no significant correlation between salivary ammonia nitrogen and blood urea or salivary urea nitrogen. There is no significant correlation between the blood urea, salivary urea, or salivary ammonia, and the DMF index or the annual caries increments.

121. Sterilization of Root Canal Instruments. *Seymour Oliet, D.D.S., School of Dentistry, University of Pennsylvania, Philadelphia, Pa.* A laboratory-clinical study was made to evaluate various methods of sterilizing root canal instruments. Tests were made on molten metal (415 to 430° F. 10 seconds), hot air (minimum 392° F. 10 seconds), and chemical "sterilization" (approximately

10 seconds). Types of hot-air sterilization tested were: "Orca" hot air sterilizer, glass beads of large and extrafine diameters, and steel ball bearings of large diameter; all used in a molten metal sterilizer. Laboratory studies were conducted by contaminating various root canal instruments with: *Strep. faecalis, E. coli, C. albicans, B. subtilis,* and *B. globigii.* Culture media used was brain-heart infusion broth with added inactivators. Clinical studies were carried out by using instruments contaminated through normal clinical usage on patients undergoing endodontic therapy. Cultures were taken before and after testing, using suitable media. The following are the preliminary results in order of efficacy: Laboratory–molten metal, glass beads (extrafine), liquid phenol, alcohol flaming, "Orca" hot air sterilizer, paramonochlorphenol, alcohol 70 per cent, tincture of Metaphen 1:200, steel ball bearings (large diameter), Camphophenique, Zephiran chloride 1:1,000, glass beads (large diameter), alcohol 95 per cent, double chlorinated soda, and sterile water; clinical–molten metal, glass beads (extrafine), liquid phenol, Roccal 50 per cent (quarternary ammonium compound), "Orca" hot air sterilizer, paramonochlorphenol, tincture of Metaphen 1:200, steel ball bearings (large diameter), and Zephiran chloride 1:1,000.

122. The Use of Paper Chromatography for the Identification of Fusobacteria. *Robert R. Omata, Ph.D., National Institute of Dental Research, Bethesda, Md.* It was reported by Jackins and Barker (*J. Bact.* **61**: 101, 1951) that oral fusobacteria can be differentiated into 2 groups by their metabolic products, including lactic, acetic, and butyric acids, formed during their fermentation of glucose. In the present study paper partition chromatography was employed for the separation and identification of the volatile fatty acids produced by 11 strains of oral fusiform bacilli, originally isolated from normal and diseased mouths. These were cultured in a medium containing Trypticase, yeast extract, and glucose. After 96 hours' incubation, the medium was steam-distilled to collect the volatile fatty acids. Adequate controls were included in all tests. After concentration, the distillates were chromatographed on Whatman No. 1 filter paper sheets and developed in n-butanol-ammonia systems at room temperature. The acids were located by spraying the chromatograms with alcoholic solution of bromeresol-green. All strains of fusobacteria studied were found to produce acetic and butyric acids from glucose. No other volatile fatty acids were found to be present in accordance with the findings of Jackins and Barker.

123. Experimental Occlusal Trauma in the Monkey. *B. Orban, M.D., D.D.S., and S. N. Bhaskar, D.D.S., Ph.D., Colorado Dental Foundation, Colorado Springs, Colo., and College of Dentistry, University of Illinois, Chicago, Ill.* To study the relationship between excessive occlusal forces and periodontal disease. MOD inlays were cemented in the upper first premolars of 4 adult monkeys. These inlays were so constructed that in the centric and the right lateral movements the premolar tooth was subjected to excessive occlusal stress. The monkeys were profused with 10 per cent formalin at 3 days, 3 weeks, 3 months, and 6 months postoperatively, jaws decalcified, and the upper premolar area was sectioned serially in a buccolingual direction and the corresponding region of the lower jaw was sectioned in a mesiodistal direction. The findings were: (1) At 3 days postoperatively the tension as well as the pressure sides showed thrombosis of periodontal blood vessels; in addition, the pressure side showed bone necrosis and crushing of connective tissue fibers. (2) At 3 weeks both the pressure and tension sides showed extensive areas of root resorption and on the pressure side there was extensive resorption of bone. (3) Three- and

6-month postoperative stages were characterized by complete repair of root surface and alveolar bone. (4) Tearing of periodontal fibers on the tension side was never observed. (5) Periodontal pockets did not develop at any stage.

124. The Influence of Sodium Chloride on the Metabolism of Glucose by Lactobacillus Acidophilus. I. Bacterial Counts. *Hubert B. Palmer, D.D.S., Norman C. Laffer, Ph.D., and John E. Faber, Jr., Ph.D., USAF School of Aviation Medicine, Randolph Air Force Base, Randolph Field, Texas, and Department of Bacteriology, University of Maryland, College Park, Md.* Batches of Rogosa's LBS medium were prepared to contain no glucose or 0.11 M of the carbohydrate. Aliquots of same were then prepared to contain sodium chloride ranging from 0.0 to 2.0 M. The sterile broth, after having been dispensed in 50 ml. quantities in milk dilution bottles, was inoculated with 9×10^3 cells (glucose-starved bacteria) of *L. acidophilus* ATCC No. 4356 and incubated for periods of 40 and 64 hours at 37° C., following which bacterial counts were made. In the study of Rogosa's broth containing no glucose it was found that as the concentration of the sodium chloride ranging from 0.0 to 6.0 M increased, the logarithm of the mean number of colonies per milliliter of broth decreased from 7.04 to 5.32 in the 40-hour cultures and from 8.65 to 5.64 in the 64-hour cultures. However, when 0.8 M of sodium chloride was present in the broth, the growth of the lactobacilli was stimulated, with the logarithm of the mean number of colonies being 5.86 and 8.88 for the 40- and 64-hour cultures, respectively. The presence of 1.3 M of sodium chloride in the broth inhibited all growth in both the 40- and 64-hour cultures. In a study of Rogosa's broth containing 0.11 M of glucose and with or without sodium chloride present the bacterial count was generally higher than similar media containing no glucose. However, here also it was observed, depending upon the concentration of sodium chloride present, whether growth of lactobacilli was inhibited or stimulated. In the glucose media prepared to contain 1.1 M sodium chloride, the growth of the organisms was inhibited by 95 per cent.

125. The Pulp-Protective Effect of Silicone Under Silicate Cement Fillings. *K. J. Paynter, D.D.S., Ph.D., G. Nikiforuk, D.D.S., M.S., and A. W. S. Wood, D.D.S., Faculty of Dentistry, University of Toronto, Toronto, Canada.* The chemical structure of silicones imparts to them a thermal stability and resistance to many types of reagents to an extent not found in most organic polymers. The ability of these agents to form water-repellent surfaces offers theoretical possibilities of protecting tooth surfaces against the action of many reagents. Thirty silicone preparations, including methyl silicone oils, alkyl silicone resins, alkyl-aryl silicone resins, and methylchlorosilanes, were tested for the following properties: (1) their ability to protect teeth against acid decalcification, (2) solubility in certain organic solvents, and (3) drying time. Testing was done as follows: standard areas, 3 mm. in diameter, were exposed through wax coverings on the surfaces of noncarious teeth. These windows were coated with a silicone preparation and dried in air for 10 minutes. The teeth were suspended in 45 ml. of a 0.5 M lactate buffer, pH 4.0 for 6 hours. The degree of etching was determined by silver nitrate staining and by measuring the amount of calcium dissolved. Of the silicones tested it was found that a preparation of a methyl-silicone resin in methyl-methacrylate monomer gave best results. This preparation was subsequently tested as to its ability to protect the rat molar pulp under a silicate filling using equipment and methods described earlier. Cavities 300 μ deep were prepared in the mesial third of the lingual surface of both the right and left upper 1st molar

teeth in a series of rats, and cavities were filled with either silicate cement alone, or with a silicone liner under the silicate cement. Animals were sacrified with ether at periods from 24 hours to 1 week postoperatively and histologic sections made of the treated 1st molar teeth. An inflammatory reaction was observed in each pulp under the fillings with no liner. No such reaction was seen in pulps of teeth under cavities which had been lined with the silicone preparation prior to filling. This indicates that the methyl silicone resin, when applied to the base of the cavities as a 30 per cent solution in methyl-methacrylate monomer, afforded protection for the pulp against the phlogogenic effect of the silicate cement fillings.

126. Ethylenediamine Kok-Glycol in the Removal of the Organic Matter of Dentin. *S. C. Peckham, B.S., and F. L. Losee, D.D.S., M.S., U. S. Naval Dental School and National Naval Medical Center, Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md.* Ethylenediamine has been used recently for the removal of the organic matter in bone (Williams and Irvine, *Science* **119**: 771, 1954). The present study compares certain extraction properties of this solvent on dentin with that of a 3 per cent solution of potassium hydroxide in ethylene glycol (Crowell, Hodge, and Line, *J. D. Res.* **14**: 251, 1934). The desiccated dentin sample used for these determinations contained 3.3 per cent nitrogen and had an ash content of 74 per cent (550° C. for 3 hours). Extraction of 5 portions of the dentin sample with an 80 per cent aqueous solution of ethylenediamine resulted in residues which consistently represented 78 per cent of the initial weight. Nitrogen concentrations of these residues ranged from 0.1 per cent to 0.2 per cent. Extraction of the dentin with KOH-glycol gave residues which ranged from 46 per cent to 55 per cent of the initial weight. Nitrogen was not detectable in these residues. KOH-glycol appears to have the advantage in extraction of organic matter, at the expense of the mineral phase.

127. A Progress Report on the In Vitro Studies of the Etiology of Dental Caries. *Ward Pigman, Ph.D., Reidar F. Sognnaes, D.M.D., Ph.D., Edward Gilman, Ph.D., Ralph Powell, B.S., and M. L. Muntz, B.S., Medical Center, University of Alabama, Birmingham, Ala., and School of Dental Medicine, Harvard University, Boston, Mass.* Lesions produced in extracted human teeth by in vitro procedures previously described have been examined at Boston by histologic examination of thin sections. Bacterial plaques were observed on the enamel surfaces. The primary penetration of enamel proceeded between prisms and was accompanied by enhancement of the prisms, cross striations, and incremental lines. Gram-positive spheroid microorganisms were found within distended dentinal tubules; in some areas these had invaded the ramifications and destroyed the intertubular matrix. Preliminary experiments have been made to determine the ability of pure strains of several oral microorganisms to attack teeth under in vitro conditions. The bacteria included several species each of oral lactobacilli, streptococci, and micrococci. All these organisms attacked the teeth, but considerable difference was shown in their ability to destroy enamel and dentin. One lactobacillus strain exhibited a marked attack on intact enamel but little attack on dentin. A streptococcus strain attacked dentin without much effect on enamel.

128. Carbonate Content of Bone, Teeth, and Francolite. *Aaron S. Posner, Ph.D., University of Liege, Belgium.* One of the major problems in the crystallography of the inorganic portion of teeth, bone, and the carbonate-bearing apatite minerals is the elucidation of their carbonate content. Some investi-

gators believe that a carbon radical substitutes in some way in a structural position in the apatite. Others maintain that the carbonate exists as a phase separate and distinct from the apatite portion of these materials. Results are presented to support the latter theory of admixture as opposed to substitution. The materials studied were the inorganic portions of human enamel and dentin, cow bone, and the mineral, francolite. The carbonate is shown to be preferentially soluble in weak HCl solutions. X-ray diffraction patterns show only the phosphate phase and display no differences in the apatite pattern before and after acid leaching. Finally, these samples were studied by infrared spectroscopy and the resulting adsorption curves were found to contain the characteristic bands of calcite and magnesite. In conclusion, it is suggested that the carbonate in these materials appears as very finely divided calcite and magnesite physically admixed with the major apatite phase.

129. The Control of the Posture of the Mandible During Rotation of the Head. *Samuel Pruzansky, D.D.S., M.S., National Institute of Dental Research, Bethesda, Md.* In the course of a study on the electromygraphic patterns of activity of the temporal and masseter muscles in normal and pathologic conditions in man, the effect of rotation of the head on the electrical behavior of these muscles was observed. In the normal subject, sitting upright, with the head postured in the Frankfort horizontal plane and the mandible at rest, no electrical activity was recorded from the temporal and masseter muscles. When the subject voluntarily rotated his head approximately 90 degrees to the right or left, while maintaining his head in the same horizontal plane, a regular pattern of low amplitude activity was recorded from the temporal muscles. Except where the exercise was performed against resistance, the masseters demonstrated little or no activity. The over-all pattern recorded by means of surface electrodes was duplicated when coaxial needle electrodes were utilized. In individuals with torticollis, action potentials of low amplitude were recorded from the temporal muscles while the patient assumed his natural, although asymmetric, posture. Since the pattern of electrical activity recorded from the temporal muscles in normal subjects and in patients with torticollis was a consistent finding, it is suggested that these physiologic phenomena are the manifestation of a reflex designed to stabilize the posture of the mandible during movements of the head. Szentagothai's demonstration of a monosynaptic reflex are for the trigeminal system offers a possible pathway for the mediation of such reflex behavior. Ultimate elucidation of this reflex should be possible through animal experimentation.

130. Experimental Exposure of the Molar Pulp of Syrian Hamsters. *Mervyn B. Quigley, H.D.D., L.D.S., R.F.P.S., Eastman Dental Dispensary, Rochester, N. Y.* This experiment was carried out to determine the reaction of the pulps of hamsters to exposure and treatment. The pulps of the first upper right molars of 10 hamsters, aged 55 days, were exposed. The exposures were covered with either $Ca(OH)_2 - H_2O$ or zinc oxide–eugenol and covered with zinc phosphate cement. The animals were sacrificed in pairs, 1 calcium hydroxide- and 1 zinc oxide–eugenol-treated animal, on the 2nd, 5th, 9th, and 14th postoperative days, and decalcified paraffin sections prepared and stained with hematoxylin and eosin. Two types of controls were kept: (A) an exposure completely untreated; and (B) the unexposed upper left first molars of each. The untreated exposure on the 14th postoperative day showed complete degeneration of the pulp. The calcium hydroxide–water-treated teeth showed areas of necrosis, well demarcated from the rest of the pulp, which was normal. The zinc oxide–eugenol-treated teeth showed no clear demarcation

and presented stages of inflammation ranging from generalized inflammation both acute and chronic, to localized abscess formations. The study showed that hamsters may be considered useful in further pulp studies.

131. Comparison of Caries Indices for Clinical Tests. *Arthur W. Radike, D.D.S., Hamilton B. G. Robinson, D.D.S., M.S., and Paul C. Kitchin, M.S., D.D.S., U. S. Air Force and College of Dentistry, Ohio State University, Columbus, Ohio.* Previous work indicates a preference for the DMF index based upon surfaces when a complete expression of caries increment during a test period is desired. However, some investigators prefer the DMF index based upon teeth. The DMFS and DMFT caries indices were compared in several ways. A study of clinical caries test data indicated that about the same proportion of the caries increment was associated with questionable caries diagnoses when either index was used. When the purpose of a test is to determine the number of caries initiations the DMFS index appears to introduce less error than the DMFT index. In grade-school children the DMFT index primarily represents caries in occlusal surfaces while the DMFS index more nearly represents new caries in all surfaces.

132. The Experimental Production of Sarcomas by Methyl Methacrylate Implants. *Irwin B. Robinson, D.D.S., M.S., Daniel M. Laskin, D.D.S., M.S., and Joseph P. Weinmann, M.D., College of Dentistry, University of Illinois, Chicago, Ill.* Methyl methacrylate film (1 cm. square; 0.008 inch thick), prepared from material similar to that used in denture construction, was implanted subcutaneously in the abdominal wall of 50 Harlan strain albino Swiss mice. As a control series, pieces of cellophane (1 cm. square; 0.004 inch thick) were implanted in a second group of 50 mice. Sections through the area of the implants from the animals which did not develop tumors showed that the polymethyl methacrylate and cellophane films become encapsulated by connective tissue. The fibrotic reaction was greatest around the cellophane. Small foci of inflammatory cells were observed in a few instances adjacent to both materials. The first tumor in relation to a polymethyl methacrylate implant was observed 257 days postoperatively. Twenty animals were living at that time. Four additional tumors subsequently occurred between 405 and 469 days. In the mice containing cellophane only 1 tumor developed, occurring 400 days postoperatively. Seven of the original 50 animals were then living. The tumors arising from both the polymethyl methacrylate and cellophane showed considerable structural uniformity and were all diagnosed as fibrosarcomas.

133. Nutrition of the Vellonella. *Morrison Rogosa, M.S., National Institute of Dental Research, Bethesda, Md.* The genus Veillonella (e.g., *Micrococcus gazogenes, Micrococcus lactolyticus*) comprises a group of small, anaerobic cocci which are present in the rumen of certain animals and also in considerable numbers in the human mouth. These microorganisms are characterized by a lack of carbohydrate fermentation. However, they possess marked biochemical activity toward lactic, succinic, and certain other organic acids. Although some information concerning the biochemical transformation of lactate by the Veillonella has been accumulated, little is known of their optimal growth requirements or their fundamental nutrition. The present study is concerned with the nutrition of the Veillonella. In an atmosphere of 95 per cent N and 5 per cent CO_2, the Veillonella have been cultured repeatedly from washed inocula (diluted at least 10^{-4} times from the original culture) in a medium adjusted to pH 8.0 before autoclaving, and comprising acid hydrolyzed vitamin-free casein, hypoxanthine, vitamins, sodium lactate, and inorganic

salts. In the foregoing medium, excellent growth after overnight incubation, equivalent to or superior to that attained in media containing yeast or other protein digests, has been observed routinely. The only vitamins indispensable for growth have been found to be thiamine and those of the B_6 group. The remaining vitamins have been inert or at best only questionably stimulatory. The amino acid nutrition of the Veillonella is presently under study. Thus far, it appears that tyrosine and serine are indispensable for growth.

134. Outline of a Theory of Periodontal Disease. *Theodor Rosebury, D.D.S., School of Dentistry, Washington University, St. Louis, Mo.* Available data on periodontal diseases (marginal and ulcerative gingivitis, periodontitis, so-called periodontosis and variants of each), although incomplete, permit formulation of a unified theory of pathogenesis. It is assumed that the teeth and supporting structures of modern man continue to be built to last a lifetime, instead of being doomed ontogenetically to early loss and phylogenetically to extinction. Periodontal disease is regarded as pathologic and as extrinsically induced and potentially preventable. It is postulated that the interrelated complex of phenomena embracing physiologic growth, maturation, and aging of the dental apparatus is subject to a range of disturbances whose sequels, varying in rate of development, severity, and reversibility, constitute periodontal disease. Integration of what is known of this physiologic process and its pathologic modifiers may be achieved without oversimplification. This theory does not point to isolated "causes" of periodontal disease. It suggests that all such diseases proceed through a characteristic mixed infection, apparently qualitatively similar in all clinical types of periodontal disease, but differing in degree and in acuity to inflammation and associated damage. These are looked upon as unifying pathologic phenomena; but infection is clearly not primary, and neither infection nor inflammation, nor "local" versus "systemic" agencies, nor the activity of any one tissue, nor any other individual component, can be assigned unique or preponderating significance. Clinical and pathologic differences among types of periodontal disease are postulated as traceable to different patterns of resistance and response to the characteristic infection.

135. A Plate Method for the Study of Collagenase Activity. *Genevieve Roth, D.D.S., and Hugh I. Myers, Ph.D., School of Dentistry, University of Kansas City, Kansas City, Mo.* In order to study the action of the enzyme collagenase on unaltered or native collagen, a plating technic has been devised. Rat-tail collagen paper as prepared by Oakley et al. requires a high concentration of the enzyme to go to complete lysis; bacterial cell debris complicates the readings, and only gross results are significant. Hidepowder coupled with an azo dye (azocoll) yields a colorimetric method for evaluation of enzyme action on the denatured or altered substrate. Collagen extracted from dentin has been incorporated into agar and well-technics employed on this denatured protein. It has been reported in the literature, and it has been found in this laboratory, that different microorganisms differ in their ability to lyse these substances. However, the unaltered collagen is considered a definitive test for collagenase activity. One gram collagen paper in 40 c.c. distilled water is ground by hand in a tissue homogenizer. Temperature is controlled by ice water in the pestle. Difco blood agar base (8 Gm. in 160 c.c. water) is autoclaved at 15 pounds for 15 minutes. It is melted and brought to 50° C. in a water bath and the collagen-water suspension is added. Collagen is altered by temperatures above 65° C.; hence it cannot be autoclaved. For this reason, the value of the plate is limited largely to testing activity and not for isolating procedures. In

some instances plates can be prepared which show no contamination. Anaerobic cultivation under hydrogen also decreases contaminants. Even when a collagenase-positive contaminant does develop, a difference in time and location of the lysis on the plate make it possible to evaluate results. Anaerobic organisms secured from periodontal lesions including Clostridial strains, as well as Clostridium from other sources, have been studied.

136. Effect of Mineral Oil as a Solvent for 9, 10-Dimethyl-1, 2-Benzanthracene. *John J. Salley, D.D.S., Ph.D., School of Dentistry, Medical College of Virginia, Richmond, Va.* In a previous communication dealing with the induction of squamous-cell carcinoma in the oral epithelium of the Syrian hamster, it was shown that acetone was a better solvent than benzene for the 3 carcinogens used because of higher tumor incidence and lower toxicity (*J. D. Res.* **33**: 253, 1954). A nonvolatile solvent (mineral oil, U.S.P., heavy) for 9, 10-dimethyl-1,2-benzanthracene decreased the tumor induction time from 6 to 7 weeks (acetone solvent) to 4½ weeks. Control animals painted with mineral oil per se exhibited no neoplasia after 50 weeks of treatment and observation. Differences between mineral oil and acetone solvents were further studied by using the animal's cheek pouch as an in vivo microscopic spread preparation so that the early stages of tumor initiation could be observed. In pouches treated with mineral oil solutions, the vehicle containing the carcinogen could be seen as droplets (5 to 7 microns in diameter) on the basal epithelial cells 24 hours after application. In 48 to 72 hours these droplets were emulsified into small particles which were distributed in the intercellular spaces of the basal epithelial cell layer. When the same hydrocarbon dissolved in acetone was topically applied and observed in vivo, there was rapid volatilization of the solvent, leaving a residue of crystalline 9, 10-dimethyl-1, 2-benzanthracene adherent to the keratinized surface of the epithelium. At no time could particles be seen inside the epithelium, as was the case with mineral oil. It appears that mineral oil as a solvent acted as a co-carcinogen on oral epithelium by causing rapid dissemination of the cancer-inducing compound in and around the epithelial cells. Less tissue deformity was also observed when mineral oil was the solvent than when volatile substances were used.

137. The Effects of Local Irritation on the Periodontium of Protein-deprived Rats. *H. C. Sandler, D.D.S., M.S.P.H., S. S. Stahl, D.D.S., M.S., and L. R. Cahn, D.D.S., F.R.C.S., Veterans Administration Hospital, Brooklyn, N. Y.* Thirty-two rats, each weighing an average of 325 grams at the beginning of the experiment, were divided into 3 groups. The 1st group was placed on a diet containing 2% protein, the 2nd group on a protein-free diet, and the 3rd group was kept on a normal stock diet to serve as a control. At various intervals during the experiment, a local irritant was placed between the lower 1st and 2nd molars of some of the animals in each group. The irritant, which consisted of a stainless steel wire, was inserted through the interproximal space below the contact point and looped around the neck of the tooth. The wire ends were twisted together and forced into the interproximal gingiva. In order to accommodate the wire, a channel had to be drilled into the adjoining walls of the teeth which led to pulp exposures in a majority of animals in each group. All animals were maintained for a maximum of 2 months, except for 7 animals in the 2% protein-containing diet group, which following deprivation were placed on a normal diet for an additional 2 months. The outstanding changes seen in the dental supporting structures of the protein-deprived animals were a degeneration and disorganization of many of the periodontal

fibers, a reduction in the number of osteoblasts and cementoblasts, and an osteoporosis of the supporting bone. A tendency toward a fatty type of marrow was also seen. The addition of a local irritant led to a marked resorption of the alveolar crest and a retardation of reparative processes following injury. This retardation of repair was seen both periapically as well as interdentally. Increase in time of experimentation as well as increased protein deprivation led to more severe changes in all animals observed. Changes following injury in protein-deprived animals seemed to be lasting, for they were seen to continue in animals which were placed on a normal diet following deprivation.

138. Dentition of Skulls of Young Precolumbian Indians. *Karl V. Sarnas, L.D.S., Eastman Dental Dispensary, Rochester, N. Y.* In cooperation with Department of Anthropology, University of Kentucky, examinations have been carried out of approximately 100 skulls. Observations have included occurrence of dental decay, condition of occlusion, tooth dimensions, palatal measurements and cephalometric analysis.

139. Relation of Salivary Lactobacillus Counts to Methyl Red Glucose Rinse Test. *Bhim Sen Savara, B.D.S., M.S., University of Oregon Dental School, Portland, Ore.* Hardwick and Manley (1952) demonstrated that 0.1% aqueous methyl red solution when sprayed on the teeth after a 1% glucose rinse turns from yellow to red if the pH changes to 5 or less. Tooth surfaces demonstrating such a color change indicate enough acid production to consider that surface area "caries-susceptible." In a group of 21 persons, glucose rinse–methyl red test was applied after obtaining saliva samples for lactobacillus counts. The data failed to show any direct association between lactobacillus count and the number of "susceptible" tooth surfaces positive to methyl red test. In another group of 55 persons, the saliva samples were obtained following the performance of the glucose rinse–methyl red application. Lactobacillus counts and methyl red positive tooth surfaces again showed no correlation. In a third group of 4 dental hygiene students 28 methyl red–glucose rinse tests and lactobacillus counts were made at different times of the day. The data showed no relationship. Lactobacillus counts were made of prebreakfast salivary samples in 23 persons but showed no association with methyl red positive tooth surfaces observed during the day.

140. Production of Hyaluronidase by Viridans Streptococci Isolated From Human Gingival Crevices. *Henry W. Scherp, Ph.D., and Stig D. Schultz-Haudt, L.D.S., School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* One hundred twenty-one single colony strains of viridans streptococci were isolated from the gingival crevices of 17 adults, selected only for the presence or absence of clinically manifest marginal gingivitis, and were tested for the production of hyaluronidase. Only strains that produced 2 turbidity reduction units or more per milliliter of broth culture were recorded as positive. All 10 subjects with gingivitis yielded 1 or more strains that formed the enzyme; only 2 of the 7 subjects with clinically normal gingivae did so. Twenty-six of the 56 strains of *Streptococcus mitis* and 7 of the 15 strains of *Streptococcus salivarius* isolated from the gingivitis group formed hyaluronidase; only 3 of the 43 strains of mitis and none of the 7 strains of salivarius isolated from the nongingivitis group did so. Also tested were 28 pooled strains from 14 additional subjects. Each consisted of from 10 to 20 single colony strains each of mitis and salivarius from a single subject. Ten of the 13 pooled strains from 6 subjects with gingivitis produced hyaluronidase; only 3 of the 15 pooled strains from 8 subjects with clinically normal gingivae

did so. All differences were statistically acceptable. These results indicate the participation in gingivitis of a more specific group of bacteria than has been generally recognized.

141. Rate of Secretion by Individual Salivary Gland Pairs in Man Under Two Conditions of Stimulation. *Leon H. Schneyer, D.D.S., Ph.D., and Lawrence K. Levin, B.S., School of Dentistry and Medical College of Alabama, University of Alabama, Birmingham, Ala.* This investigation is concerned with determining, simultaneously or in close consecutive order, the rates of secretion by the individual submaxillary, sublingual, and parotid gland pairs of man under 2 conditions of stimulation. The modified Pickerill "segregator" has been employed for the collection of separate submaxillary and sublingual secretions (Schneyer, 1954). Under conditions of reduced exogenous stimulation, i.e., essentially "resting" conditions, it is found that the submaxillary glands contribute the major part of the total secretion derived from the 3 pairs of large salivary glands (approximately 70% of this "fraction total"; 23 subjects). A smaller percentage (approximately 25%) is derived from the parotid glands. Only about 5% of the fraction total is contributed by the sublingual glands. Under conditions of increased exogenous stimulation, obtained by application of dilute acetic acid to the tongue, the submaxillary glands continue to produce the greatest (63%; 16 subjects), the sublinguals the smallest (2.8%), and the parotids an intermediate part (34%) of the fraction total. The fraction total itself makes up about 55% of the total mixed saliva. The remaining 45% possibly is largely derived from small mucosal glands.

142. Production of Chondrosulfatase by a Microorganism Isolated From Human Gingival Crevices. *Stig D. Schultz-Haudt, L.D.S., and Henry W. Scherp, Ph.D., School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* An enzyme capable of depolymerizing chondroitin sulfate was produced in all of 10 mixed broth cultures of gingival bacteria obtained from 10 subjects with chronic marginal gingivitis, and in 8 of 11 such cultures from 11 individuals with clinically normal gingivae. The search for microorganisms producing chondrosulfatase was concentrated on the mixed broth cultures from 4 of the subjects having gingivitis. These cultures were streaked out on Bordet-Gengou sheep blood agar and incubated under a mixture of 95% nitrogen and 5% carbon dioxide at 37° C. for 24 hours. It was found that mixed colonies consisting of a staphylococcus and a gram-positive rod were capable of producing chondrosulfatase in beef extract broth. These colonies were streaked on Bordet-Gengou agar, and after 72 hours of anaerobic incubation tiny, light yellow secondary colonies of the gram-positive bacilli appeared on the top of the white colonies of staphylococci. These could be picked and the bacilli subsequently grown as isolated colonies on the medium under the conditions mentioned. The organism was tentatively classified as a microaerophilic diphtheroid. When grown in beef extract broth aerobically 15 of 17 single colony strains tested produced chondrosulfatase after 24 hours at 37° C. Anaerobically, all single colony strains tested (16) produced the enzyme, which was exocellular and constitutive. Chondrosulfatase was not produced by alpha and beta streptococci, staphylococci, aerobic diphtheroid bacilli, and fusiform bacilli from human gingival pockets.

143. The Microstructure of Chimpanzee Teeth Compared With Those of Other Anthropoids, Man and Monkey. *E. L. Schuman, D.D.S., and R. F. Sognnaes, D.M.D., Ph.D., Department of Anthropology and School of Dental*

Medicine, Harvard University, Boston, Mass. By means of a replica technic, we have demonstrated the presence of various enamel defects, micropits and irregular laminated perikymata, similar to those found in man, in the teeth of noncaptive chimpanzee, gorilla, and gibbon. Histologic ground sections were prepared from a relatively large sample of chimpanzee teeth and from a few gorilla and gibbon teeth. The pattern of incremental lines of enamel and variations in dentin structure, interglobular and areolar dentin, which are common in the teeth of man, were also found to be present in the anthropoids in a moderate degree, whereas the teeth of noncaptive monkeys are characterized by either an absence of or very slight evidence of these conditions.

144. Urinary Fluorine Levels Following the Clinical Use of a Stannous Fluoride-Containing Dentifrice. *Richard A. Schweinsberger, M.A., and Joseph C. Muhler, D.D.S., Ph.D., Department of Chemistry, Indiana University, Bloomington, Ind.* To determine if a stannous fluoride-containing dentifrice (F = 1000 ppm), which was previously shown to reduce the incidence of dental caries in children (*J. D. Res.* **33**: 606, 1954) increases the urinary fluorine level in children residing in a low-fluorine area (water fluorine level, 0.1 ppm), the amount of fluorine in the urine was analytically determined in a group of boys who used the fluoride dentifrice for a minimum of 18 months. These data were compared to data from an identical group using a similar dentifrice not containing fluorine. Since it is generally accepted that children consuming 1.0 ppm fluorine in the communal water supply from birth have no toxic manifestations, it was of interest to compare the urinary fluorine levels of children using the stannous fluoride dentifrice with similar children who use a communal water supply with a natural fluorine concentration of 0.85 ppm. The data clearly indicate that there is no difference between the urinary fluorine level of either group using the dentifrices in regard to fluorine concentration or total fluorine, while there is approximately a 4-fold increase in both the concentration and total amount of fluorine in the urine of those children residing in the fluoride area. These data indicate that the use of this fluoride-containing dentifrice does not contribute to any significant increase in the urinary fluorine level of children. Furthermore, since the urinary fluorine level is considered to be quite sensitive to fluoride ingestion, these data indicate that the children using the fluoride dentifrice are well below any level of ingestion suggesting chronic toxicity.

145. Possible Caries Prevention by Establishing in the Mouth Certain Antagonists to Oral Acidogenic Microorganisms. *Charles A. Scrivener, D.D.S., College of Physicians and Surgeons, San Francisco, Calif.* Twenty-five different strains of microorganisms which are antagonistic to oral lactobacilli have been isolated from saliva and classified. Bacilli, cocci, and filamentous forms are represented. Many of the microorganisms that produce antibiotics and are not ordinarily found in the saliva have also been tested. It has been our hope to induce the predominate growth in the mouth of these organisms. Efforts to inoculate the various antagonists in large numbers into the mouth have previously failed because they disappeared almost as rapidly as they were introduced. The adaptation of microorganisms presented a possible solution. A strain of *Staphylococcus albus* isolated from saliva and ATC #10068 *Bacillus brevis* were selected because of their potent anticaries properties. A series of slant culture tubes was prepared by adding dehydrated culture medium in diminishing proportions and 2% agar to homogenized saliva. Saliva was used in place of the usual distilled water for mixing the medium. The recommended amount of brain-heart infusion was added to the 1st group of tubes. Five per cent

less nutriment was added to each succeeding group of the series so that the last group of culture tubes contained only saliva and agar. The selected organisms were transferred after 48 hours' growth in each group to the successively "reduced nutrient" groups. In this manner the organisms were induced to survive on the nutritive substances present in saliva alone. Tests indicated that no toxic properties had been acquired. These organisms have survived in the mouth several months after inoculation. Mass inoculations of these organisms in the mouths of students are being undertaken. Statistical analyses will be utilized to determine the possible caries reduction in the inoculated group in relation to a control group.

146. The Influence of Hormones on Rat Salivary Glands. *William G. Shafer, D.D.S., M.S., and Joseph C. Muhler, D.D.S., Ph.D., School of Dentistry, Indiana University, Indianapolis, Ind., and Department of Chemistry, Indiana University, Bloomington, Ind.* A general investigation of the effect of hormones on the rat salivary glands has been continued. The procedures studied have included gonadectomy, adrenalectomy, hypophysectomy, and the administration of estradiol, diethylstilbestrol, testosterone, methylandrostenediol, desiccated thyroid, and thiouracil. Except for hypophysectomy, the administration of thyroid extract and thiouracil have produced the most severe alterations in the structure of the submaxillary glands. These changes were specifically manifested in the granular tubules and appeared as a variation in their size. In hypophysectomized males, the average tubule diameter was 27.6 microns (2.0),* while control males average 40.8 microns (1.3). Hypophysectomized females averaged 28.1 microns (2.2), control females 37.8 microns (2.0). Thyroid-treated animals exhibited a marked significant increase in the size of tubules: males: 47.1 microns (1.3), compared with controls 39.3 microns (2.2); females: 45.2 microns (3.5), compared with controls 40.0 microns (1.2); while thiouracil-treated animals showed a significant decrease in the size of tubules: males: 35.6 microns (1.1), females 30.4 microns (1.3), compared with the same controls. The changes in the submaxillary glands of the rats treated by any of these procedures are nonspecific but may be related to primary variations in thyroid activity. In the majority of studies, a correlation existed between experimental caries incidence and the structure of the submaxillary gland.

147. Comparative Studies on the Caries-Susceptibility of Ten Strains of the Common Laboratory Rat. *James H. Shaw, Ph.D., Harvard School of Dental Medicine, Boston, Mass.* Representatives of 10 strains of the common laboratory rat were maintained throughout the reproductive cycle on a cariogenic purified diet (Shaw, *J. D. Res.* **26**: 47, 1947). Six of these strains were obtained from the Wistar Laboratories; 1 was obtained from the Holtzman breeding colony; the remaining 3 had been maintained in our laboratories for numerous generations. Their offspring were maintained on the same purified diet throughout their entire lifespan, including 1 or more reproductive cycles. Offspring in the second generation were tested for their caries-susceptibility by maintenance on 3 caries-producing diets: this purified diet, the Hoppert-Webber-Canniff diet, and McClure's cooked cereal diet. These diets gave the same relative results for the caries-susceptibility of the strains, but uniformly the purified diet caused the greatest amount of tooth decay and the cooked cereal diet least with the Hoppert-Webber-Canniff diet intermediate. The strains with the highest caries-susceptibility were the Harvard susceptible, and the Wistar mutant albino strains. The Wistar waltz-whirler, the Wistar

*Standard deviation.

chocolate, and the Holtzman strains were moderately susceptible. The Wistar yellow red-eye, the Wistar yellow blue-eye, and the Wistar fawn were moderately resistant strains. The Long-Evans and Harvard resistant strains were the most caries-resistant of the 10 strains. The most homogeneous results were obtained with the Harvard susceptible, the Harvard resistant and the Long-Evans strains which for several generations have been selected for particular characteristics of caries incidence. Within each remaining strain, there was a much higher degree of variability in dental caries experience.

148. Effect of Sodium Fluoride on Oral Lactobacilli Isolated From the Rat. *Tetsuo Shiota, Ph.D., American Dental Association Research Fellowship, National Institute of Dental Research, Bethesda, Md.* The effect of sodium fluoride on the rate and extent of growth of 20 homofermentative and 20 heterofermentative oral lactobacilli isolated from rats was studied. The rate of growth of strain 84AT, a typical homofermentative lactobacillus, was decreased by the addition of sodium fluoride in the range of 50 to 200 ppm in broth cultures. At the next higher concentration tested (300 ppm), an induction or lag period was apparent in the growth curves. After 48 hours' incubation, with all strains the amount of growth with concentrations of NaF from 50 to 500 ppm was proportional to the pH of the medium. In bactericidal experiments the fraction of cells killed increased as the pH of the testing medium was lowered. At 50 ppm NaF, the group represented by strain 84AT showed a decrease in the amount of growth, whereas the group represented by strain pp3 did not. However, the heterofermentative lactobacilli did not appear to be fluoride-resistant. Additional experiments are being conducted to determine whether the fluoride-resistant lactobacilli are biochemically distinct from the nonfluoride-resistant type.

149. The Composition of Calcified Tissues. *Leon Singer, B.S., M.S., Ph.D., and W. D. Armstrong, B.A., Ph.D., M.D., The Medical School, University of Minnesota, Minneapolis, Minn.* An analysis of the major constituents of dense cortical bone has been made. A comparison of values obtained as a result of several preparatory treatments for the removal of protein in the tissue is given. Considerable variation in composition is reported when the tissue is ashed at high temperature, boiled in potassium hydroxide–ethylene glycol, extracted with ethylene diamine, or shaken with 0.165 M sodium chloride.

150. Comparison of Lactobacillus Counts and Acid Production in Selective Culture Media. *Marshall L. Snyder, Ph.D., and Margery K. Gardner, B.S., University of Oregon Dental School, Portland, Ore.* In evaluating laboratory tests for estimation of caries activity, we have been interested in the relative merits of culture media proposed for lactobacillus counts and acid production in selective culture media. Specimens (3,666) of saliva routinely collected from children under study were cultured on tomato-juice peptone agar (pH 5.0) containing 1:10,000 sodium azide and Rogosa's SL agar. It was apparent that the concentration of azide accounted for the somewhat lower counts on the TJA as compared with those on SLA, since this differential was lost when the concentration of azide was reduced to 1:20,000. The selective action of the azide on yeasts and molds was still retained. No essential difference was observed between the rate of positive reactions (acid production) in Snyder test agar and Rogosa's SLA (mod) for colorimetric purposes compared with each other and lactobacillus counts on either TJA containing 1:10,000 azide or Rogosa's SLA. It was concluded that investi-

gators could, with due allowance for the 1:20,000 concentration of sodium azide in TJA, choose freely between any of these media for lactobacillus counts and/or acid production in selective culture media.

151. Postnatal Changes in Proteolytic Activity and in the Morphology of the Submaxillary Gland in Male and Female Albino Rats. *Leo M. Sreebny, D.D.S., Ph.D., Julia Meyer, B.S., Erica Bachem, B.S., and J. P. Weinmann, M.D., College of Dentistry, University of Illinois, Chicago, Ill.* Five developmental stages were studied: early and late prepuberal rats (15 and 30 days of age); early and late puberty (60 and 90 days of age); and young adult animals (150 days of age). The submaxillary gland was examined histologically from a quantitative morphologic point of view, and an assay of its proteolytic activity was made. Postnatal changes in the glands consisted in a progressive increase in specific proteolytic activity, which was fastest during puberty. This increase in enzymatic activity could in part be correlated with an increase in the amount of intralobular duct tissue per unit volume, and with an increase in the granule content of these ducts. The specific proteolytic activity was found to be higher in male than in female rats, from the beginning of puberty on. In the rat, as in the mouse, the submaxillary glands are the site of secondary sex differences. Although these differences find no discernible structural expression in the rat, male and female animals are nevertheless found to differ functionally. The submaxillary gland of male rats has a greater capacity for protein breakdown, both by virtue of its greater size and of its higher specific activity, than that of the females.

152. Pulpal Changes Resulting From Freezing Storage Technics. *C. E. Staley, D.D.S., and D. E. Waite, D.D.S., College of Dentistry, State University of Iowa, Iowa City, Iowa.* Recent dental literature has reported the apparent success of both autogenous and homologous transplantation of teeth. These successes suggest the storage or establishment of "tooth banks," which would greatly increase the availability of selected teeth for transplantation. Developing teeth, usually the 3rd molar or premolars, have been prepared by both fast and slow methods of freezing. The basic technics available for this type of procedure are: (1) iso-pentane, which is probably the fastest method of freezing (–150° C.); (2) dry ice-pentane-slush, also a very fast method (–79° C.); (3) liquid nitrogen, considered a slow method but, due to its extremely low temperature, the least chemical activity is permitted (–196° C.); (4) dry ice, the slowest method, but usually the most convenient (–70° C.). The reasoning behind the freezing technic for storage of teeth is the same as it is for all types of living tissue. The tooth as tissue is difficult to study because it is composed of both soft and hard tissue. This offers a problem when freezing and rewarming, because of the differential coefficient of thermal expansion of the two tissues. For example, when freezing with any of the faster methods, cracking of the enamel occurs; but by using dry ice in alcohol slush this did not occur. Prior to freezing, the teeth are subjected to various concentrations of glycerol. This aids in the removal of water from the pulp. This technic on the human tooth was guided by results first obtained by using the mature bovine tooth. All teeth were then sectioned and studied microscopically.

153. Early Cytologic Changes in Experimentally Induced Tumors of Rat Submaxillary Salivary Glands. *Samuel M. Standish, D.D.S., School of Dentistry, Indiana University, Indianapolis, Ind.* Early cytologic changes fol-

lowing implantation of 3-methycholanthrene-"Carbowax" pellets into the submaxillary salivary glands were observed on weanling and adult rats (153 animals) of the Sprague-Dawley strain. Animals were sacrificed at 1, 2, 4, 8, 12, 16, 20, and 24 weeks following implantation. Controls were: (1) sham-operated animals to determine the possible effect of incision and repair, and (2) pure "Carbowax" implanted animals to determine the effect of the carrier upon the salivary glands. No significant alteration of parenchymal architecture other than connective tissue repair was noted in any control. Carcinogen-implanted animals exhibited a desmoplastic reaction at all sacrifice intervals. Approximately 83% of all animals, in which the implant was found on histologic section to be in the substance of the submaxillary gland parenchyma, demonstrated some degree of ductal metaplasia. Squamous metaplasia of ductal epithelial origin was observed at all sacrifice intervals with a significant increase in degree at the 12-week period. Since this metaplasia appeared to originate from striated duct epithelium, no pertinent differences could be established in the histogenesis of induced tumors in weanling and adult rats. The responses obtained included connective tissue proliferation, ductal metaplasia, central abscesses, epidermoid cysts, keratin cysts, fibrosarcomas, and epidermoid carcinomas.

154. Anatomic Limitations in Intraoral Roentgenography of the Upper Molar Region. *Russell G. Stephens, D.D.S., and John H. Barr, D.D.S., Tufts College Dental School, Boston, Mass.* Placement of films parallel to the long axes of the teeth has advantages in locations where it is practicable. Clinical experience has indicated that the anatomy of the upper arch seriously limits the effectiveness of the parallel-film technic. The upper molar region was selected for study, for here the technic would offer its greatest advantages. The reduced vertical angulation possible with a parallel film avoids superimposition of the malar shadow and presents a true orientation of anatomic structures. The 2 limiting factors in this region are the height of the palate vault and the height of the malar bone, from the occlusal plane. Measurements were recorded for 100 subjects between 19 and 33 years of age. The data were analyzed statistically. Mean palate heights opposite the 6- and 12-year molars were 19 and 18.5 mm., respectively, mean malar heights in the same regions were 25 and 26 mm., and the average upper molar tooth lengths are 21 and 20 mm. (Black). These results show the palate vault to be considerably lower than suggested by the illustrations used in the literature on the technic. There is no correlation of any predictive value between malar height and palate height. Charts prepared from the data reveal the inability of the parallel-film (right-angled projection) to produce routinely an acceptable film, that is, a film unobscured by the malar shadow and showing at least 2 mm. of periapical bone. At an axial inclination of 5 degrees, success could be predicted in 13 cases or 13% of the total and in 4% of the total cases at 10 degrees. At any greater angulation the malar shadow would be superimposed. Using all possible angulations of radiation, and assuming axial inclinations of 5, 10, 15, 20, and 25 degrees, an unobscured view could be produced in 24, 24, 27, 37, and 38 per cent of the total cases, respectively. The study indicates that the parallel-film has limited usefulness in the upper molar region due to anatomic incompatibilities.

155. Evaluation of an Antihistaminic in Oral Surgery. *Lucian Szmyd, D.M.D., Dental School, Georgetown University, Washington, D. C.* The double-blind technic with 500 representative oral surgery patients was used to determine the effect of an antihistaminic on postoperative sequelae in oral surgery.

Only local anesthesia was utilized. Untoward reactions produced by the antihistaminic as well as its effect on blood pressure were studied. Statistical analysis of the results was based on arcsine transformation of percentage counts and chi square test, assuming that the effect of antihistamine is no different from that of placebo. The probabilities obtained were: vomiting 0.30, nausea 0.026, drowsiness 0.00004, insomnia 0.56, dryness of mouth 0.22, headache 0.76, weakness 0.31, nervousness 0.48, blurred vision 0.48, osteitis alveolar 0.72, neuralgic pain 0.60, incidence of hemorrhage 0.80, swelling degree 0.90, swelling duration 0.85, trismus degree 0.85, trismus duration 0.90, pain degree 0.45, pain duration 0.50, diastolic pressure 0.90, systolic pressure 0.70. Statistical analysis shows that nausea is significantly reduced while drowsiness is significantly increased by antihistamine as compared with placebo. There is no statistical evidence that vomiting, insomnia, dryness of mouth, headache, weakness, nervousness, blurred vision, osteitis alveolar, neuralgic pain, hemorrhage, swelling, pain, trismus, diastolic pressure, and systolic pressure were effected beneficially or harmfully due to antihistamine as compared with placebo.

156. Quantitative Characterization of Bacterial Interaction by the Cross-Titration Method. *Duane F. Taylor, M.S.E., and Theodor Rosebury, D.D.S., National Bureau of Standards, Washington, D. C., and School of Dentistry, Washington University, St. Louis, Mo.* One phase of a continuing research into interactive phenomena among indigenous microorganisms by means of the cross-titration method (Rosebury, Gale, and Taylor, *J. Bact.* **67**: 135, 1954) has been the further analysis and simplification of the quantitative aspects of the method. A nomograph has been devised whereby the observed pattern of inhibition, the maximum effector (inhibiting) organism concentration, and the maximum test (inhibited) organism concentration may be combined directly to yield numerical values of slope of the front of inhibition and effective inhibiting concentration for each test plate. This eliminates the necessity for constructing the special slide calculator previously required. The cross-titration method has been analyzed in terms of the general mathematical problem of interpreting continuous phenomena by means of observations taken at a limited number of points. The limit of accuracy inherent in the method is considered with special attention to the number and size of the dilution intervals employed on a single plate.

157. Studies on Oral Filamentous Organisms. *John C. Thonard, B.D.Sc., L.D.S., Basil G. Bibby, B.D.S., Ph.D., D.M.D., and Thomas G. Ludwig, B.D.S., M.S., Eastman Dental Dispensary, Rochester, N. Y.* The present study was undertaken to find more suitable media for the growth of the filamentous bacteria commonly found in the mouth and after several subcultures in both liquid and solid media to separate them into 3 broad categories of actinomyces, fusiforms, and leptotrichia. Morphologic, cultural, and biochemical descriptions of the types will be given and the characteristics of an organism which we believe previously undescribed will also be presented. This bacterium has been tentatively placed in the genus leptotrichia but has a tendency to bacillary formation. No conclusions regarding the possible importance of these organisms will be drawn at this stage.

158. The Effect of Diet on the Calcium Concentration of the Milk of Lactating Rats. *Svein U. Toverud, D.M.D., and Paul L. Munson, Ph.D., Harvard School of Dental Medicine and Harvard Medical School, Boston, Mass.*

A 30% decrease in milk calcium concentration 2 days after imposition of a purified low-calcium diet (Shaw) has been observed in lactating rats suckling their first litters (Munson, Toverud, and Kenny, *J. D. Res.* **33**: 676, 1954). The possibility that the change in milk calcium concentration was merely a concomitant of a change in water content can be eliminated on the basis of results obtained in a new series of experiments on lactating rats with 2nd litters. Eight days after changing to the purified low-calcium diet the milk calcium had declined an average of 31% in rats prefed laboratory chow, and there was no significant change in water content. Other rats were prefed the purified diet, except that 1.5% calcium was added, and a significant decline in milk calcium was likewise found 8 days after changing to the low-calcium diet. Thus, a decrease in milk calcium can be brought about without change in the diet except for a reduction of the calcium content. In the rats with 2nd litters, the decrease in milk calcium did not occur within 2 days following the change in diet, as observed previously with primiparous rats, and it is possible that age or previous lactational experience may have made these rats more resistant to calcium restriction in the diet.

159. The Structure of the Cementum of Mammalian Tusks. *Otto R. Trautz, Ph.D., and Emma Fessenden, B.S., College of Dentistry, New York University, New York, N. Y.* Various mammals carry tusks which are modified incisor or canine teeth, remarkable for their persistent growth. Cross sections of these tusks show the presence of mantle layers which appear distinctly different from the underlying orthodentin. In the tusks of the elephant and the narwhal these layers reach a thickness up to 3 mm. and are more translucent than the dentin. X-ray diffractograms disprove the suggestion that they may be a type of enamel, as the diffraction lines are not sharp, as from the crystallites of enamel apatite, but the lines are diffuse as obtained from dentin, cementum, and bone. In textbooks these layers are described as cementum. However, similar to the enamels, the apatite crystals show a high degree of preferred orientation. The crystallites are grouped into 2 fiber structures (*J. D. Res.* **32**: 420, 1953): most have their *c* axes more or less parallel to the surface and the length direction of the tooth, the others have a more or less radial direction. There is no fiber structure with a tangential direction. Similar to previous experiences (Trautz, *Ann. Dent.* **12**: 47, 1953), the fiber structures of the apatite are oriented parallel to the collagen fibers. Examination of the thin sections in polarized light shows the orientation of the collagen fibers due to their birefringent effect: we find chiefly longitudinal and fewer radial and no tangential fiber bundles. The orientation of the collagen fibers determines also the distinct mechanical properties of this cementum layer, which are evidenced by fractured elephant tusks. A surface layer of similar structure is found on the walrus tusk and the sperm-whale tooth and probably on all tusks with persistent growth which are not covered with enamel.

160. Toxicity Studies With Six Local Anesthetics. *Paul E. Tullar, Ph.D., Dental Branch, University of Texas, Houston, Tex.* This study was undertaken as one of a series of comparisons dealing with the toxic natures of local anesthetics in common use in dentistry. Adult white mice of uniform age, size, and sex were selected for the study because of their greater sensitivity to respiratory and cardiac depressants, and because of the ease and simplicity with which deaths from these causes can be differentiated. Aqueous solutions of the soluble salts were injected intraperitoneally to insure optimum absorption in the face of possible vasoconstrictor effects as well as to avoid erratic absorption times peculiar to subcutaneous administrations. LD_{50} doses were established from group mortalities above and below the LD_{50} level. Of

the compounds tested, piperocaine, procaine, and lidocaine produced LD_{50} values above 100 mg. per gram of body weight; while tetracaine, Ravocaine, and dibucaine gave LD_{50} values below 100 mg. per gram of body weight. Relative toxicities ranged from 1.3 to 12.9 times that of the least toxic. Respiratory failures accounted for deaths in each instance; and occurred within 12 minutes of injection.

161. A Histochemical Study of Protein-Bound Sulfhydryl and Disulfide Groups of Normal and Inflamed Human Gingiva. *Samuel Turesky, D.M.D., Jeanne Crowley, B.S., and Irving Glickman, D.M.D., Tufts College Dental School, Boston, Mass.* The distribution of protein-bound sulfhydryl and disulfide groups was studied in normal and inflamed human gingiva using the Barrnett-Seligman histochemical technic. Specimens of clinically normal and inflamed gingiva were obtained from 35 patients, 15 to 71 years of age. Hematoxylin and eosin-stained sections were used for orientation. In the epithelium sulfhydryls and disulfides were demonstrable between the cells, as well as in the cytoplasm and to a lesser degree in the nucleus. While in the normal gingiva the concentration of sulfhydryl groups increased from the basal layer to the stratum corneum, there appeared to be a uniform distribution of disulfide groups throughout the epithelium. In chronic gingivitis there was a diffuse diminution of sulfhydryls and disulfides associated with degenerative changes in the epithelial cells. In the connective tissue, sulfhydryls and disulfides were present in the fibroblasts and endothelial cells, and in minute amounts in the intercellular substance of both normal and inflamed gingiva, and in the leukocytes of inflamed gingiva.

162. The Inhibition of Calculus Formed In Vitro. *G. Van Huysen, D.D.S., and V. Bhatavadaker, B.D.S., School of Dentistry, Indiana University, Indianapolis, Ind.* Calculus formed on dissociated teeth in paraffin-stimulated saliva renewed twice a day can be detected microscopically with polarized light at the end of 1 week. A 0.5% solution of mucinase (Prolase 100 and Mylase L1 in saliva definitely reduced calculus formation as well as saliva odor.

163. The Influence of Certain Plaque Adsorbed Agents on Hamster Caries. *J. F. Volker, D.D.S., Ph.D., L. D. Apperson, Ph.D., and W. J. King, Ph.D., School of Dentistry, University of Alabama, Birmingham, Ala., and Colgate-Palmolive-Peet Co., Jersey City, N. J.* Certain compounds, which exhibit retention on protein and inhibition of acid production by salivary organisms, were investigated for their effect against hamster caries. The materials studied were penicillin, sodium-N-lauroyl sarcosinate, and sodium p-hydroxy mercuric benzoate in powder, paste, and aqueous solution, respectively. Two different dietary routines were employed. In 1, the nursing animals were permitted access to a cariogenic diet on or about the 10th day of life. At approximately the 22nd day, they were weaned and continued on the diet for an additional 45 days before being sacrificed. In the other, the animals were denied access to the diet until after normal weaning on the 26th or 27th day of life. Then they were placed on the diet for an additional 90 days, at which time they were sacrificed. Of the compounds tested, penicillin was the most effective, sodium-p-hydroxy mercuric benzoate showed no effect, and sodium-N-lauroyl sarcosinate gave a small effect against hamster caries. Differences between the effect of sodium-N-lauroyl sarcosinate against acid production by hamster and human saliva were noted. Differences were also noted in the pH of the salivas: 8.8 to 9.2 for hamster saliva as compared with 6.6 to 7.0 for human saliva tested under the same conditions. It is suggested that these differences be taken into account in interpreting effects of suggested caries reducing agents when used in hamster studies.

164. Effect of Varying Concentrations of Sodium Fluoride on Parasitic and Free-living Protozoa and Rotifera. *Wayne W. Wantland, Ph.D., Department of Biology, Illinois Wesleyan University, Bloomington, Ill.* Parasitic protozoa in the intestinal tract of golden hamsters are diminished in numbers when the host is given ad libitum 2 to 10,000 ppm NaF in drinking water. These parasites disappear from the intestine of hamsters when higher concentrations of NaF (0.5 c.c. 500 to 10,000 ppm) are administered by stomach tube daily for 5 days. In vitro experiments indicate a strong resistance of these parasitic protozoa to 2 to 100 ppm NaF. On microscopic slides concentrations of 500 to 10,000 ppm NaF prove lethal to these parasites in from 10 to 40 minutes. Free-living protozoa (Paramecia and Euglena) and freshwater forms of Rotifera continue to be alive and active when placed in NaF concentrations of 2 to 1,000 ppm but are killed rather quickly when placed in 1,700 to 10,000 ppm NaF. Using Rotifera and Paramecia as test organisms and a modification of the Hellige Fluoride Reagent No. 611-S751 (MHR), a biologic test requiring a period of 2 hours has been devised which has accurately differentiated between water containing 10 ppm NaF (4.5 ppm F) or less and water containing 20 ppm NaF (9.5 ppm F) or more. Data presented (1) provide strong evidence against the idea still prevalent in many areas that fluorine is a highly toxic protoplasmic poison even in low concentrations, and (2) further promote testing procedures for confirming amounts of fluorine added to waters which should markedly allay the fears of the most critical and apprehensive individuals.

165. The Metabolism of Microconcentrations of Different Fluorides in Rats. *Donovan A. Weddle, B.S., and Joseph C. Muhler, D.D.S., Ph.D., Department of Chemistry, Indiana University, Bloomington, Ind.* This study was designed to compare the metabolism of NaF, Na_2SiF_6, and SnF_2 in rats when a stoichiometrically equivalent amount of fluorine is given in the drinking water at a concentration of 1.0 ppm. The fluorine ingestion was controlled by giving measured, limited amounts of the respective fluoride solution to each animal. When all the fluorine-containing water had been consumed by each animal, redistilled fluorine-free water was available ad libitum. The total fluorine excreted by each animal was determined, in the feces and urine separately, for each 3-day period of the experiment, and the total F retained by the animal was determined by ashing the entire carcass. One group received sodium fluoride and retained 1.83 mg. F, excreted 0.61 mg. F in the urine, and 0.13 mg. F in the feces. The second group, which received sodium silicofluoride, retained 1.63 mg. F, excreted 0.50 mg. F in the urine, and 0.14 mg. F in the feces. The third group of animals received stannous fluoride and retained 1.50 mg. F excreted 0.42 mg. F in the urine, and 0.13 mg. F in the feces. These data indicate that the fluorine administered as sodium fluoride is more readily absorbed from the gastrointestinal tract than is sodium silicofluoride and is thus more available for incorporation into the apatite structures.

166. Rate of Formation and Structural Quality of Experimental Reparative Dentin in Molar of Rat. *Stanley R. Weider, Isaac Schour, and Clive I. Mohammed, University of Illinois, College of Dentistry, Chicago, Ill.* Experimental cavities were prepared in the upper 1st molars of 88 young adult white rats. In 68 animals, the cavities were filled with zinc oxide and eugenol, Aquadont, and Aquadont containing 50 per cent tricalcium phosphate. In the remaining 20 animals; the cavities were left open. Specimens were prepared after a survival period of 2 and 3 weeks and sections were cut with the freezing microtome. Following a latency period of approximately

1 week, the daily rate of formation of reparative dentin averaged from 3 to 8 microns. The rate decelerated with time (age gradient), increased with the depth of the cavity, and varied with the type of filling material. At 21 days, the average daily rate for medium cavities was approximately 3.5 microns for Aquadont and for zinc oxide and eugenol; 6 microns for Aquadont containing 50 per cent tricalcium phosphate; and 8.5 microns for open cavities. The quality of the dentin structure was scored on the basis of the regularity of the tubules, the degree of calcification, and the presence or absence of cellular inclusions. The structural pattern showed 3 zones, the acute calciotraumatic layer and the initial and later zones of formation. It was related to its rate of formation so that the portion deposited initially at a higher rate was relatively poor, but improved as the rate decelerated. The quality of the dentin also decreased with the cavity depth and with the degree of irritation of the filling material.

167. Alterations in the Dental Tissues Following Beryllium Carbonate Feeding. *Frank M. Wentz, D.D.S., Ph.D., Chicago College of Dental Surgery, Loyola University, Chicago, Ill.* The effects on the upper rat incisor of feeding 3 and 6% beryllium carbonate have been studied in 81 white rats during a 14- to 168-day period. The beryllium carbonate was fed as a supplement to uniform synthetic diets low in vitamin D (300 units per 100 Gm. of diet) and diets high in vitamin D (2,670 units per 100 Gm. of diet). When the beryllium carbonate-fed rats received minimal doses of vitamin D, severe rickets developed as the blood phosphorus fell to 2 mg. per cent. When the animals were fed maximal doses of vitamin D in a diet containing beryllium carbonate, only subclinical signs of rickets were observed, and blood phosphorus levels were normal. The animals with severe rickets showed enamel hypoplasia and aplasia; severe retardation in dentin formation and calcification, retardation, and finally, arrest in eruption. The changes were progressive to 70 days. Partial recovery occurred in older animals. In the animals with subclinical rickets, enamel formation and maturation were not disturbed; dentin formation and calcification were slightly retarded; and eruption was normal.

168. The Effect of Cations Above Hydrogen in the Electromotive Series on Experimental Caries in the Syrian Hamster. *Joel Wisotzky, D.D.S., and John W. Hein, D.M.D., Ph.D., School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* In reviewing the literature for the effects of cations on experimental rat and hamster caries, we noted that a possible relationship might exist between the position which a cation occupies in the electromotive series, and the action of that cation as an inhibitor or noninhibitor of dental caries. We have made the working hypothesis that, in general, cations above hydrogen in the electromotive series will not inhibit, while oxidizing cations found below hydrogen in the electromotive series should inhibit experimental dental caries. To test this hypothesis, we conducted caries tests on hamsters, selecting 4 cations with positions above hydrogen (Mg++, Li+, Cd++, and Pb++) and 4 cations with positions below hydrogen (Cu++, Au+++, Pd++, and Pt++++). The tests on the cations above hydrogen have been completed, and the results are as follows: (a) 1 meq. Li++ as $LiSO_4$ gave a 10% increase in male caries and a 17% decrease in female caries; (b) 1 meq. Mg++ as $MgSO_4$ gave a significant 39% increase in male caries and a significant 53% increase in female caries; (c) ½ meq. Cd++ as $CdSO_4$ gave an 11% increase in male caries and a 99% increase in female caries; (d) ½ meq. Pb++ as Pb $(C_2H_3O_2)_2$ gave a significant 38% increase in male caries and a 56%

increase in female caries. Due to the toxicity, the ½ meq. Cd solution was changed to a ¼ meq. solution in the middle of the experiment. In conclusion, the cations above hydrogen seem to have run true to prediction when tested under similar experimental conditions. There were no caries inhibitors among the group, which contained 2 in vitro acid inhibitors, and the definite trend was toward caries acceleration.

169. Cariogenicity of a High Sugar Ration as Affected by Variations in the Calcium-Phosphorus Ratio. *Winfrey Wynn, M.A., John Haldi, Ph.D., Mary Louise Law, A.B., and Katherine Bentley, A.B., Division of Basic Sciences in the Health Services, Emory University, Emory University, Ga.* Quadruplicate littermate albino rats of the Wistar strain were desalivated at weaning and placed on 4 high-sucrose rations which were of the same composition except that the Ca:P ratios were adjusted so that in Ration 1 the ratio was 2:1, in Ration 2, 1:1, in Ration 3, 1:2, and in Ration 4, 1:3. The adjustment was made by the addition of a neutral sodium phosphate mixture, leaving the calcium content of 0.5% constant in all 4 rations. The phosphorus content of the Rations 1 through 4 were 0.25, 0.5, 1.0, and 1.5 per cent, respectively. The animals were fed ad libitum for a period of 80 days after which time they were sacrificed and the teeth scored for caries. There were 40 rats on each ration. The average number of lesions on Rations 1 through 4 were 22, 18, 13 and 11, respectively, and the average caries scores were 44, 25, 16, and 13. These results indicate that the cariogenicity of a high sugar ration when fed to albino rats may be modified by changes in the Ca:P ratio.

170. Further Studies on the Effect of Subminimal Amount of Magnesium on the Syrian Hamster: Extraction Wound Healing. *George M. Yamane, D.D.S., School of Dentistry, University of Minnesota, Minneapolis, Minn.* Syrian hamsters, 40 days old, were separated according to sex and divided into 8 groups of 4 animals each; 4 experimental groups (Ia, IIa, IIIa, and IVa) receiving 40 ppm magnesium, and 4 control groups (Ib, IIb, IIIb, and IVb) receiving 647 ppm magnesium. Animals in Groups Ia and Ib were sacrificed 8 days after extraction; Groups IIa and IIb, after 13 days; Groups IIIa and IIIb, after 17 days; and Groups IVa and IVb, after 23 days. Under pentobarbital sodium anesthesia, the right and left lower first molars were extracted, one side being used for paraffin sectioning and the other side for ground sectioning to demonstrate new bone formation by means of alizarin red S, which had been injected intraperitoneally at the time of extraction, and again, 36 hours before sacrifice. Grossly all the sockets of the experimental and control animals, except those in Group Ia, were covered by normal-appearing gingival epithelium. In the case of the experimental animals in Group Ia, a probe could be inserted to the depth of the socket. Histologically there was no evidence of bone formation in the experimental animals until the 13th day after extraction. On the 13th day, there was bone formation at the lateral wall and the fundus of the socket of the experimental animals. This condition was somewhat similar to that seen in the sockets of the control animals of Group Ib, which were sacrificed 5 days earlier. Thus, magnesium appears to play an important role in the healing of the extraction wounds of molars of the Syrian hamster.

171. A Method for Studying the Epithelial Attachment and the Gingival Sulcus. *Helmut A. Zander, M.S., D.D.S., School of Dentistry, University of Minnesota, Minneapolis, Minn.* Clinical measurements can be correlated with histologic observations by a simple reproducible method. A film

material, like cellulose acetate or Mylar, is placed into the gingival sulcus. After the specimen is obtained, this film material can be either left in for sectioning or dissolved out before sectioning. The film strip can be seen in the microscopic section, and its relation to the histologic structures can be observed. Dog, monkey, and human specimens will be presented to show how this method sheds light on the relation of the epithelial attachment to the clinical sulcus between the tooth and the gingival tissues.

172. Antibacterial Activity of Mixed and Parotid Human Saliva: A Preliminary Report. *Bernard J. Zeldow, M.S., School of Dentistry, University of Pennsylvania, Philadelphia, Pa.* Antibacterial substances in saliva contribute to the natural defense mechanisms of the oral cavity. Growth of lactobacilli and other oral bacteria is inhibited in vitro by many human salivas. Antibacterial activity was tested by placing mixed or parotid salivas in wells cut in culture medium inoculated with *Lactobacillus acidophilus* ATCC #4357. Growth was inhibited. Subcultures made from the zones of inhibition did not contain viable bacteria. The inhibitory component is contained in the supernatant of centrifuged saliva; it is stable when heated at 56° C. for 5 minutes, but is inactivated after 5 minutes at 75° C. Concentration by lyophilization resulted in increased zones of inhibition. Parotid saliva was collected (Lashley apparatus) from 11 subjects; bacterial counts were at least 99% lower than for mixed saliva. The growth inhibitory activity of parotid was compared with that of mixed saliva of the same subjects; the activities were not found to be consistently different, indicating that the inhibitory component was not of bacterial origin. Blood serum of 12 subjects whose saliva contained antibacterial activity did not inhibit the test organism. These findings suggest that the inhibitory component is a product of the salivary glands.

173. Deposition of Fluorine in Various Tibial Segments of the Young Growing Rat. *I. Zipkin, Ph.D., and R. O. Scow, M.D., M.S., National Institute of Dental Research and National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, U. S. Department of Health, Education and Welfare, Bethesda, Md.* Twenty-four 20-day-old male Sprague-Dawley rats were divided equally by weight into a control and a fluorine-treated group. A stainless steel pin was inserted into the left tibia of each rat, and the treated group received a total of 3.15 mg. F intraperitoneally over a period of 31 days. The increase in length of the tibial diaphysis at each end was determined by measuring the distance from the proximal end of the pin to the proximal and distal epiphyseal lines on roentgenograms taken at 20 and 51 days of age. Upon sacrifice, 4 segments of tibia were obtained for analysis, namely, the proximal epiphysis (PE), the proximal diaphyseal (PD), the middle diaphyseal (MD), and the distal (D) segments. The proximal diaphyseal segment represented bone formed during the experimental period, and the middle diaphyseal segment represented that portion of the diaphysis present at the beginning of the experiment. Pooled samples of the segments were analyzed for ash and fluorine. The per cents of ash (oven-dry basis) of PE, PD, MD, and D segments of the fluorine-treated rats were 30.9, 44.3, 57.3, and 35.9, respectively, and did not differ significantly from those of similar segments in the control rats. Fluorine also had no effect on the growth rate of the various tibial segments. The per cents of fluorine in the ash were 0.132, 0.097, 0.098, and 0.129, respectively. The newly formed bone (PD) had the same capacity for fluorine deposition as the old bone (MD), but both these diaphyseal segments had a lower fluorine concentration than the proximal epiphysis. In addition, no relation was apparent between the per cent ash and the fluorine concentration in the various tibial segments.

IV. Abstracts of Papers Presented to the Dental Materials Group

M-1. Improved Phonetics in Artificial Denture Construction. *L. R. Allen, D.D.S., M.S., School of Aviation Medicine, Randolph Air Force Base, Tex.* The purpose of this investigation was to incorporate a procedure into artificial denture construction which would enable the patient to speak clearly at the time of insertion. To determine which areas of alveolar ridge and hard palate were normally contacted in pronouncing a given consonant, a series of palatograms was made on a group of dentulous individuals with normal speech. A well-adapted artificial palate was constructed for each subject. This palate was dusted with nonscented talcum powder, carefully inserted, and the subject asked to pronounce the consonant to be studied. The contact area is evident by absence of powder. This area was outlined with a glass-marking pencil, and the tracing filed for future study. It was found that while no 2 persons contacted the same area in pronouncing a given consonant, a sufficient similarity existed to constitute a pattern. These patterns were used as a guide in making tongue palatal tracings on the artificial denture. When the maxillary denture was in the "try-in" stage, the waxed palatal portion was dusted with nonscented talcum powder, and a palatogram made. The tracing thus obtained was compared with the normal pattern, and this area was contoured, retraced, and recontoured until a normal tongue palatal contact was developed. It was found that when the pattern was normal, the speech was normal.

M-2. Hygroscopic Technic for Inlay Casting Using Controlled Water Addition. *K. Asgarzadeh, M.S., School of Dentistry, University of Michigan, Ann Arbor, Mich.* Previous studies on the hygroscopic expansion of dental casting investment have shown that different amounts of water added to the investment are proportionally related to the resulting expansion. Furthermore, a point, known as the critical point, is reached, beyond which further water addition will not increase the hygroscopic expansion. A compensating technic based on these factors has been presented previously. The purpose of this paper is to present an evaluation of this technic on the basis of practical dental castings. The versatility of this technic is demonstrated by showing the fit of a series of castings when varying the amount of water added. Differences of 0.2 c.c. of added water show differences in casting fit. Another advantage of this technic is the fact that varying manipulative conditions does not appreciably influence the expansion, provided the amount of water added is below the critical point. Castings made when varying water/powder ratio of mix as well as number of spatulations indicate that these variables are not effective in altering the casting fit when using the controlled water-added technic. In addition, the reproducibility of this technic is demonstrated by a comparison of a series of castings all made using the same amount of water added.

M-3. A New Restorative Material. *R. L. Bowen, D.D.S., San Diego, Calif.* The purpose of this report is to describe gross experimental studies of the use of an epoxy resin as a binder for fine particles of fused silicon dioxide and porcelain. This investigation demonstrates that the following combined properties may be obtained in a dental filling material: thermal expansion equal to that of tooth structure; adhesion to tooth structure after immersion in water 24 hours; color stability; and apparent stability and insolubility in the oral environment. Other properties of the mixture are discussed and compared with commonly used dental materials. X-ray transmission may be equated to tooth structure by the careful use of heavier atomic-weight elements in the filler. Abrasion resistance and crushing strength are related to the hardness of the filler particles. Shrinkage during cure (after gelation) is less than 1%. Thermal

and electrical conductivity are very low. The toxicity of the epoxy resin is low. The curing agents used thus far have been either acidic or basic and thus skin contact should be prevented. Within a very short time after mixing, the curing agent and epoxy resin have copolymerized to the extent that toxicity becomes negligible. Further investigation and development of this approach to dental restoration materials is indicated.

M-4. Sulfinic Acid Derivatives as Accelerators in the Methyl Methacrylate Polymerization. *G. M. Brauer, Ph.D., and F. R. Burns, B.A., National Bureau of Standards, Washington, D. C.* The use of sulfinic acids to promote the rapid polymerization of methyl methacrylate has been reported by Hagger, but the poor storage stability of these compounds is a serious deterence to their application in dental resins. Recently Bredereck and co-workers (*Makromolekulare Chem.* **12**: 100, 1954) have suggested aminosulfones and sulfinic acid salts as accelerators. In the present investigation amine salts of p-toluene sulfinic acid were studied. These compounds are synthesized on addition of amine to the ether solution of the sulfinic acid. Some of the compounds prepared have not been reported in the literature. Freshly prepared salts of secondary and tertiary aromatic amines such as the methyl aniline or dimethyl-p-toluidine salt are highly effective accelerators in the benzoyl peroxide initiated polymerization, but yield colored products. Aliphatic amine salts, as well as the free acid, give colorless products, but the setting time is increased considerably, especially with increasing chain length. Heterocyclic amines were not efficient accelerators. The storage stability of the amine salts appears to be better than that of the free acid, although most of the compounds slowly decomposed on storage. Polymerization studies of other sulfur-containing compounds such as alpha-amine sulfones and the speeding up of the polymerization by alcohol also will be discussed.

M-5. Studies on the Bonding of Resin-filling Materials to Dentin Surfaces. *M. G. Buonocore, M.S., D.M.D., W. Wileman, B.D.S., D.M.D., M.S., and F. Brudevold, D.D.S., M.S., Eastman Dental Dispensary, Rochester, N. Y.* By modifying the method of polymerization of a commercially available cavity sealing material we have been able to produce a resin which bonds strongly to both "wet" and dry flat dentin surfaces of extracted teeth. Acrylic materials easily adhere to this tooth-bonded interface material, thus producing a means of bonding resin-filling materials to the dentin surfaces. In contrast with complete loss of adhesion on water immersion with standard filling materials, adhesive bonds of the order of 20 kg. per sq. cm. are present after 90 days' immersion. Treatment of the dentin surface with H_3PO_4, for from 1 to 2 minutes prior to placement of the adhesive, results in high initial forces (50 kg. per sq. cm.) but these bonds suffer a rapid loss of strength under water, approaching the values obtained on surfaces not acid-treated. Using HCl for the surface treatment, bond strengths of the order of 60 kg. per sq. cm. are obtained which resist water immersion up to 30 days. Immersion to 120 days results in about ½ original strength. In testing the strength of the bond, after placement of the acrylic filling material over the adhesive coating it was generally the bond between adhesive and acrylic material that broke. We are, therefore, actually measuring the tensile strength of the bond between acrylic and coating material, not the bond existing between adhesive and dentin, which must be stronger.

M-6. A Study of the Proximal Contour of Class II Amalgam Restorations Using Various Matrix Band Technics. *C. Castaldi, D.D.S., and R. W. Phillips, B.S., School of Dentistry, Indiana University, Indianapolis, Ind.* Class II cavities have been prepared in molar and bicuspid teeth duplicated in chrome

alloy from natural teeth of the three representative types, square, tapering, and ovoid. The cavities are packed with silver amalgam alloy and the contours of the resulting restoration are studied by means of silhouette photography. The following matrix band technics have been studied: ivory #8, ivory #1, sigveland, tofflemire, adjustable band (Swiss), compound supported band (Miller technic), and compound supported band (Markley technic).

M-7. Variations in the Properties of Self-curing Acrylic Resins. *H. J. Caul, B.S., and W. T. Sweeney, B.A., National Bureau of Standards, Washington, D. C.* The temperature rise during the polymerization of self-curing resins appears to have a direct bearing on the amount of residual monomer in the polymerized material. By using a direct-filling resin polymer, and monomer with varying amounts of promoter, direct relationships have been found between promoter concentration, temperature rise, time of peak temperature, indentation strength, yield strength, and modulus of elasticity.

M-8. The Setting Mechanism of Zinc Oxide and Eugenol Mixtures. *H. I. Copeland, Jr., D.D.S., G. Brauer, Ph.D., and A. F. Forziati, Ph.D., National Bureau of Standards, Washington, D. C.* The setting mechanism of zinc oxide–eugenol mixtures is shown to be a combination of chemical and physical processes. The hardened mass resulting from mixes of zinc oxide and eugenol consists of zinc oxide embedded in a matrix of long, sheathlike crystals of zinc eugenolate. The excess eugenol is adsorbed by both the zinc eugenolate and the zinc oxide. Further investigations on the solubility of zinc eugenolate show that the material is insoluble in commonly used solvents. The molecular weight, found cryoscopically in an acetamide solution, shows that the empirical formula of the crystalline reaction product is $(C_{10}H_{11}O_2)_2Zn$, zinc eugenolate. Further infrared absorption studies give increased evidence of the chelate structure for zinc eugenolate. The setting phenomenon is demonstrated by a mixture of zinc oxide with salicylaldehyde, a known chelating agent which does not have a phenolic group. The presence of water, particle size of ZnO, and pH are important factors in the setting reaction.

M-9. Tensile Strengths of Modified Methyl Methacrylates and Various Rates of Loading. *J. Cornell, M.S., K. Stone, and S. Ellis, A.B., H. D. Justi & Sons, Inc., Philadelphia, Pa.* The disadvantages of single-speed tensile tests are presented. The design and details of operation of a variable-speed tensile tester are described. The data are developed to show the effect of cross-linking on tensile strength and the effect of water on tensile strength of various plastics. A comparison is drawn to the standard transverse strength tests used for denture base materials. The authors postulate that this type of machine will permit the testing of plastics at rates more comparable to mouth conditions.

M-10. Porosity in Dental Gold Castings. *C. W. Fairhurst, S. F. Kozak, and G. Ryge, D.D.S., Marquette University, School of Dentistry, Milwaukee, Wisc.* The relative quantities of porosity were determined by microscopic inspection of specimens prepared by metallographic procedures. A controlled casting technic was developed which included standardization of position of pattern in the ring, investing technic, pattern size (1.5 mm. thickness), temperature of gold, temperature of mold, and melting atmosphere. The variables studied are the length and diameter of sprue, temperature of mold and gold alloy, and methods of melting the alloy. As of Nov. 15, 1954, 200 castings have been made. The findings are: when gold is melted in a reducing atmosphere and castings are made using 18, 14, and 10 gauge sprues of 3 mm. length and cast into a mold of 1,292° F., only the castings made with a 10 gauge sprue were free of shrink-

age porosity and porosity from occlusion of gases. When the length is increased to 6 or 9 mm., shrinkage porosity occurs in all the castings; however, in greater quantities in castings with small sprues. When the mold temperature is reduced to 900° F. or 500° F., shrinkage porosity increases and border or surface porosity occurs. When the alloy is melted with a natural gas and air mixture, considerable porosity due to occlusion of gas was found in all castings. Technics using other gases for melting the alloy are being studied.

M-11. Spectrophotometric Characterization of Light-induced Changes in Direct-filling Resins. *T. E. Fischer, D.D.S., B. G. Butt, D.D.S., M.S., J. Sartore, M.S., and B. Eichel, D.D.S., USAF School of Aviation Medicine, Randolph Field, Tex.* Three commercial resins, Kadon, Replica, and Fasterown were selected for special evaluation following exposure to infrared, visible, long-wave ultraviolet (366 mμ) and short-wave ultraviolet (254 mμ) light. Specimens were prepared by polymerizing unpigmented resins in thin sheets and absorption spectra determined from 700 to 330 mμ with a Beckman model DR recording spectrophotometer. No change was detected following infrared and visible light irradiation in any of the resins. No change in Fasterown was detected following long-wave ultraviolet light irradiation after 7 days' exposure. A small regular increase in absorbance, however, was seen in Kadon from 580 to 340 mμ, and in Replica from 550 to 390 mμ after as little as 24 hours' radiation. Short-wave ultraviolet light irradiation resulted in a gradual increase in absorbance in Kadon from 530 to 400 mμ becoming marked in the near ultraviolet (330 mμ). Replica behaved similarly. Fasterown, however, showed marked absorption below 450 mμ with an absorption peak at 342 mμ after as little as 1 hour's exposure. The family of curves at the 342 mμ absorption peak yielded a straight-line function when absorption was plotted against the logarithm of time. The appearance of the absorption component in the near ultraviolet range probably reflects a different reaction occurring within the resin than that produced by the long-wave ultraviolet light.

M-12. Quantitative Craze Test for Dental Plastics. *J. E. Fox, B.S., and J. O. Semmelman, The Dentist's Supply Co., New York, N. Y.* Quantitative data are being accumulated on the craze-resistance of plastics, using a modification of the American Dental Association denture base flexure test. This includes a general study of the effects of various solvents in combination with applied stresses on typical dental resins. The role of surface polish and conditioning is investigated. The applied stress technic appears to afford a quantitative measure of craze-resistance and also establishes the relationship between crazing and mechanical strength loss.

M-13. Literature Abstracting and Indexing. *J. F. Glenn, B.S., L. D. Caulk Co., Milford, Del.* The problem of keeping up with the publication of technical literature is a very real one that faces every investigator. A procedure is described using a dictating machine to facilitate the rapid preparation of abstracts by the researcher. Two methods of indexing and filing literature abstracts thus made are outlined: (1) through the use of edge-punched, hand-sorted cards for the personal file, and (2) the machine-sorted card for more general or institutional use.

M-14. Wear Testing Equipment for Synthetic Resin Teeth. *A. R. T. Greenwood, D.D.S., Dental Materials Group, Australia.* Acrylic resin teeth have been frequently criticized as being too soft for use in artificial dentures, but the amount of wear assessed clinically does not appear to be as great as might be

expected from the relatively low hardness. Following quantitative measurements of wear experienced in practice by various types of patients, equipment was constructed to simulate the wear in the mouth. Whole teeth were used and the conditions arranged so that there were excursions of 1 tooth over the other in all directions but without rotation. The teeth were rubbed together under a load but without the use of any abrasive. Results showed that the hardness of acrylic teeth was a significant factor when used against natural or porcelain teeth but not when opposed to acrylic teeth. The apparatus as constructed and used produced in, 24 hours, wear of about the same degree as that found in 12 months of clinical use. The clinical experiments with acrylic teeth opposed to natural teeth showed a wide variation in the rate of wear of the former, depending to a great extent upon the vigor of the patient. Such a combination is contraindicated if the natural teeth have sharp cusp formation or show evidence of much attritional wear, and in any case should only be used under conditions of balanced occlusion.

M-15. A Study of the Antibacterial Properties of Plastic Filling Materials. *N. O. Harris, D.D.S., M.S., and T. E. Fischer, D.D.S., USAF School of Aviation Medicine, Randolph Field, Tex.* In an attempt to reduce the possibility of recurrent caries following the insertion of direct-filling resins, various antibacterial agents have been added to selected polymers. The eventually polymerized materials have then been tested for antibacterial properties, and for marginal bacterial percolation characteristics. A method was devised for the in vitro testing of the longevity of such antibacterial activity, and for the correlation of same with parallel in vivo data. Similarly, a rapid, reproducible, and simple method was designed for testing for the combined effect of the coefficient of expansion and of the antibacterial properties upon marginal bacterial percolation. These experimentations demonstrated that the more soluble antibacterial agents acted to minimize the effects of bacterial percolation. However, due to said solubility, there was a rapid loss in the potency of the drug action. Agents of lesser solubility had little effect upon the microbial population utilized in the test.

M-16. Rotating Diamond Instruments—An Evaluation. *J. L. Hartley, D.D.S., and R. B. Moore, B.S., National Bureau of Standards, Washington, D. C.* A continuing study of rotating diamond instruments has utilized the weight-loss method to evaluate their comparative cutting effectiveness. Pyrex glass rods and human enamel were cut under controlled conditions of load, speed, and time. The results of the experiments utilizing human enamel could not be correlated with those performed in Pyrex. Cutting procedures in Pyrex glass resulted in a complete breakdown of the diamond instrument in 15 minutes or less, while the breakdown point was never attained in enamel although tests as long as 190 minutes were made. Diamond instruments which had been discarded after use under clinical conditions were examined. Failure appeared due to diamond loss at the point or area of the instrument which was most frequently involved in the cutting operation. These instruments must be operated under conditions of light load and high peripheral speed for maximum life and cutting effectiveness. The low peripheral speeds of small specially shaped diamond instruments, when operated at normal speeds of rotation, result in increased applied force and consequent loss of diamonds from the areas subjected to the greatest pressure. Thus, there is breakdown of critical areas of the instruments even when used in enamel. In order to evaluate the ability of a diamond instrument to perform satisfactorily under clinical conditions, it is essential to have a breakdown test capable of measuring the ability of the bonding material to retain the dia-

monds. Proper bonding will enable the instrument to retain its diamonds even though subjected to the low peripheral speeds and high-loading encountered in routine operative procedures.

M-17. The Influence of Various Design Factors on the Performance of the Inverted-Cone Bur. *E. E. Henry, Ph.D., and F. A. Peyton, D.Sc., School of Dentistry, University of Michigan, Ann Arbor, Mich.* This report describes a program, undertaken at the Physical Research Laboratories, which had as its objective a systematic experimental study of peripheral cutting by the inverted-cone bur. The No. 37 bur was selected as being representative of this family of burs. This report deals with such variables as (1) the effect of end flutes, (2) the effect of changes in bur diameter, (3) the effect of changing the cutting length of the bur, (4) the effect of varying the number of cutting edges or flutes, and (5) the effect of varying the rake angle of the bur. Results describing the effect of these variables on (1) the ability of the No. 37 bur to cut ivorene and brass, and (2) the cutting life of the bur are presented. Although this study was experimental, and only experimental results are presented, it was guided by a general theoretical solution to the problem.

M-18. Use of Ionization Chambers in Obtaining Data on Radiation at Points Within the Head During Dental X-ray Exposure. *D. C. Hudson, D.D.S., and J. W. Kumpula, National Bureau of Standards, Washington, D. C.* A study to determine levels of ionizing radiation at various points in and about the human head during dental radiography has been undertaken. A phantom of tissue-equivalent wax was built around an adult human skull, and measurements were made using small Sievert ionization chambers placed at the skin and within the skull at 10 different locations. Radiation absorbed was measured during full-mouth intraoral dental radiography using conventional 8 inch skin target distance, 65 K.V. and 10 Ma., with 1.5 mm. of aluminum filtration, and exposing 14 films. Dosage at the skin of the cheek amounted to 20 to 25r and various lower values were obtained at other points within the head. A comparison of these radiation levels was made with measurements taken at the same points in the head when using a panoramic extraoral radiographic technic. Maximum dosage in this technic occurred at the skin of the neck and a reading of 0.450r was recorded there during full-mouth exposure. Levels within the skull ranged downward to zero from this value. Radiation absorbed at several different points in the head during dental radiography is demonstrated to be considerably less when using a panoramic method than when the conventional dental radiographic technic is employed. The technic used in these experiments and data obtained in a series of measurements will be presented and discussed.

M-19. A Study of the Effects of Casting Variables on the Mechanical Properties of Base Metal Dental Alloys. *C. Ingersoll, B.S., Williams Gold Refining, Buffalo, N. Y.* An investigation of casting variables and heat treatment was conducted on tensile specimens using tensile properties as a basis for evaluation. Variables included specimen and casting design, atmospheres, and postcasting treatment. Specimen design included 0.090 inch straight rod, 0.090 inch section with #12-24 threaded ends and 0.090 inch section with ¼—20 threaded ends. Casting design included positive flow where metal flowed directly into the specimens and negative flow where the metal flowed into a runner, reversed direction, and flowed into the specimens. Several riser sizes were investigated. Two casting methods were used. Atmospheres investigated were air, argon, vacuum, and vacuum partially relieved with argon. Postcasting treatment included air cooling, furnace cooling, water quenching, and salt bath tempering. Rubber wheel effects were evaluated. A statistical study of the results indicate

that both casting design and specimen design affect mechanical properties and porosity of specimens. Risers had no effect due to specimen design. Vacuum casting was inferior, other atmospheres equivalent. Induction melting was superior to torch melting. Heat treatment of the specimens after casting affected properties. Mechanical properties were not affected by the use of the rubber wheel.

M-20. A Twenty-eight-month Clinical Evaluation of an Acrylic Cement. *J. F. Johnston, D.D.S., Indiana University, School of Dentistry, Indianapolis, Ind.* This is a second-year report on a clinical evaluation of an experimental resin cement. Approximately 800 cemented units have been observed.

M-21. A Method of Comparing the Wear Resistance of Various Materials Used for Artificial Teeth. *S. Jordan, D.D.S., J. A. Cornell, M.S., S. Ellis, A.B., E. E. Rose, D.D.S., H. D. Justi & Sons, Inc., Philadelphia, Pa.* The fact is brought out that no standard test for the wear resistance of artificial teeth is available to research groups, and the need for one is discussed. A brief review of the literature and unsuccessful methods of testing wear resistance of teeth is presented. The authors set forth their concepts of the requirements of a good test. The complete description is presented of the wear-testing machine developed by the authors in their laboratory and considerable data are presented on various types of artificial tooth materials.

M-22. A New Dental Porcelain With Extreme Resistance to Thermal Shock. *P. W. Lee, Dentist's Supply Co., New York, N. Y.* For over a century dental porcelains have been compounded with a feldspar base. While feldspathic porcelains are quite satisfactory in most respects, they are sensitive to rapid changes in temperature and it is recognized that care must be exercised in grinding so that localized heat is not developed sufficiently to cause a check or crack. In certain dental applications where considerable grinding must be done (tube teeth), a porcelain with better grinding characteristics is very desirable. Such a porcelain has been developed using fused silica as a low-expansion basic material. This porcelain has been subjected to service trials for several years and the successful results of such work can now be reported and demonstrated.

M-23. X-Ray Diffraction Studies of Mercury Diffusion and Surface Stability of Dental Amalgam. *J. A. Mitchell, D.D.S., G. Dickson, M.S., and I. C. Schoonover, Ph.D., National Bureau of Standards, Washington, D. C.* An investigation was made of the x-ray diffraction characteristics of hardened dental amalgams under several conditions. The effects of exposure to excess free mercury, the effect of various polishing technics, and the effect of elevated temperatures upon the diffraction pattern of the hardened mass were studied. The authors' previously reported method of revealing the presence of uncombined mercury was adapted to this study. Using this method it was possible to demonstrate the ability of uncombined mercury to diffuse through hardened amalgams. The release of mercury from the structure of the set amalgam was shown to occur when it was heated to 65° C. or above or when polishing technics were employed which produced excessive heat. Uncombined mercury was found to exist in fully hardened amalgam only under these conditions. In all cases such uncombined mercury disappeared on further aging at 37° C. by combination or recombination with the other phases present.

M-24. Observations of Hunter-Schreger Bands. *J. F. Mortell, D.D.S., and F. A. Peyton, D.Sc., School of Dentistry, University of Michigan, Ann Arbor, Mich.* Hunter-Schreger bands are described generally as being an optical

manifestation of the direction in which the enamel rods have been sectioned relative to their axes. It is of interest that the widely reported interpretation of the Hunter-Schreger band phenomenon is not confirmed by the findings of the present study. Freshly extracted teeth were ground various distances pulpally from the mesial, distal, buccal, or lingual aspect of the crown. When the required depth was reached the surface was polished, etched with 0.1 N HCl, dried, and coated with a collodion preparation. The replicas so obtained were shadowed and examined. Hunter-Schreger bands were consistently found on shadowed replicas of polished etched enamel surfaces. Differences in the direction of the rods were not found in alternating bands, contrary to what has been reported. Hunter-Schreger bands do not always start at the dentinoenamel junction but may form concentric arcs in cuspal areas. The replica technic demonstrates Hunter-Schreger bands in a degree of clarity not previously reported.

M-25. Periapical X-ray Film Survey. *R. J. Nelsen, D.D.S., and R. J. Fanning, National Bureau of Standards, Washington, D. C.* A preliminary evaluation of intraoral x-ray film showed that there is a considerable variation in the characteristics of film available for the use of the dental profession. A survey of dental roentgenographic technics and films has been carried out in an effect to determine the various characteristics of periapical x-ray films. Properties studied in the survey included relative speeds of all the currently available brands of periapical films, color of the film base, thickness of the film base, degree of water resistivity of the individual film wrapper, aging characteristics, and also the sensiometric characteristics of the various brands of films.

M-26. The Effects of Peroxide, Amine, and Hydroquinone in Varying Concentrations on the Polymerization of Polymethyl Methacrylate Slurries. *E. E. Rose, D.D.S., J. Lal, Ph.D., and R. Green, M.S., H.D., Justi & Sons, Inc., Philadelphia, Pa.* A rather complete study is presented showing the effect on polymerization rate of varying, 1 at a time, the concentrations of inhibitor, promoter, and catalyst. The method of recording time and temperature in the polymerizing mass is described. Data are presented for several concentrations of each of the 3 ingredients showing the time and the temperature effects observed. The effect of several peroxides on the polymerization rate of a standard slurry is shown. The effect of particle size on the polymerization rate of polymethyl methacrylate (activated by standard amounts of promoter and catalyst) is shown.

M-27. A Method for Measuring the Mucosal Surface Contour of Impressions, Casts, and Dentures. *N. W. Rupp, D.D.S., National Bureau of Standards, Washington, D. C.* At present there is no convenient method for measuring the mucosal surface contour of impressions, casts, or dentures. A report is presented on a practical technic based on the comparative measurements of identical areas of two precisely positioned surfaces. With a dial indicator the dimensional change between the two surfaces is determined. This difference can be measured over the entire area of a denture to an accuracy of ± 0.001 inch in less than an hour's time. With this method it is possible to make the following determinations: (1) relative accuracy of impressions; (2) growth or recession in the mouth as recorded on casts; (3) effect of different technics or materials on the fit of dentures.

M-28. Hygroscopic Expansion. *G. Rygg, D.D.S., and C. W. Fairhurst, School of Dentistry, Marquette University, Milwaukee, Wisc.* The findings of this investigation are: Liquids other than water (glycerine, alcohol, transformer oil, etc.) cause "hygroscopic" expansion when introduced during the initial

setting period. X-ray diffraction studies of the hydration of calcium sulfate, hemihydrate, and of investments show that although the rate of hydration is different under normal and hygroscopic setting conditions, the final intensities reached are the same. This holds true whether the liquid added to produce the hygroscopic expansion is water or some other liquid. It is concluded that hygroscopic expansion is caused by physical factors: absorption due to capillary action, and adsorption and film formation on the surfaces of the individual particles (gypsum crystals as well as inert material) X-ray diffraction data and dimensional change determinations indicate that the hygroscopic expansion takes place before the normal setting expansion. The variation in hygroscopic expansion as caused by various liquids is explained as resulting from different surface tension and viscosity of the liquids, and from the effects of these liquids on solubility and crystal habits. The explanation offered will account for the findings of this investigation as well as the observations published by other researchers in this field.

M-29. Dental Resin Cements and Direct-Filling Resins Advocated for Posterior Teeth. *P. J. Schouboe, B.S., and G. Paffenbarger, D.D.S., National Bureau of Standards, Washington, D. C.* Modified methyl methacrylate resin is being used as a luting and cementing medium in dentistry to fasten appliances and restorations to teeth. Because these cements adhere fairly well to hard tooth tissues when dry they are also advocated as filling materials. An investigation of physical properties of these materials shows the following results: (1) setting time range of 4 ½ to 15 minutes; (2) solubility in H_2O and compressive strength of the same order as zinc phosphate cements; (3) radiopacity generally lower than zinc phosphate cements; (4) film thicknesses as low as 10 microns; (5) volumetric expansion from 0.7 to 2.0 per cent on sorption of water; (6) poor color stability; (7) no adherence to tooth structure under conditions of use; and (8) optical opacities ranging from 45 to 100 per cent on 1 mm. thick specimens. The resins are principally of the acrylic family and the polymers (powders) are heavily loaded with mineral fillers. Preliminary studies on other properties such as thermal expansion, shrinkage on polymerization, solubilities in various solutions, hardness, elastic limit, and flow, and on composition will be reported.

M-30. A Method of Measuring Porosity in Porcelain Teeth. *J. O. Semmelman, Dentist's Supply Co., New York, N. Y.* With the introduction of vacuum firing, it has become possible to secure near perfect density in dental porcelain. Corresponding improvements are thus required in the technic of porosity measurement. Previous gravimetric methods were laborious and of relatively low accuracy. An optical method was devised in which a specimen (which may be an actual porcelain tooth) is ground and polished as it would be in normal service. Observation with a filar head microscope permits a count and size measurement of the exposed pores or bubbles and a calculation may be made of percentage porosity by area or volume. Application of metallographic polishing technics improves accuracy and results in data, such as the following:

TECHNIC	BUBBLES PER SQ. MM.	AVERAGE MAXIMUM DIAM. MICRONS	PER CENT POROSITY
Normal porcelain–air-fired	62.2	61.0	6.34
Vibrated porcelain–air-fired	92.2	28.9	1.81
Normal porcelain–vacuum-fired	5.9	27.6	0.11

M-31. A Study of Rotational Speed Used in Cavity Preparation. *E. W. Skinner, Ph.D., School of Dentistry, Northwestern University, Evanston, Ill.*

Operating speeds for diamond stones and steel burs, selected by a dental practitioner in his general practice, were determined using a tachometer attached directly to the dental engine. The optimum speeds for the different tools, as judged by the dentist, were determined empirically.

M-32. Ultrasonic Method of Jacket and Veneer Crown Preparation—Preliminary Report. *P. M. Steen, D.D.S.* With the advent of ultrasonics into the field of dentistry, many interesting and provocative uses of this means of tooth cutting are unfolded. One of these phases is the preparation of jacket and veneer crowns with the use of the Cavitron ultrasonic dental instrument. Due to the fact that this instrument vibrates about 30,000 times per second (ultrasonic), a vast amount of the noise, pain, and vibration of the conventional drill is lost. These vibrations in turn agitate aluminum oxide particles which flow in a water solution over the tip of the instrument, and which in turn do the actual cutting of the tooth (molecular excavation) by extreme conformity with the cutting tip, of which there are many types. The jacket crown preparation is done with 4 or 5 different tips and is accomplished in 6 definite and precise steps. The average conventional preparation requires approximately 30 minutes. With the ultrasonic instrument about the same length of time was required for the preparation plus the fact that a smooth, polished finish was imparted to the preparation making further polishing unnecessary. Some slight sensation was noticed by a few patients when the dentoenamel junction was crossed. In 1 or 2 of these cases it was handled comfortably with a small amount of local anesthetic. However, the most singular aspect of the ultrasonic jacket preparation in all cases handled to date, is the great loss of noise, vibration, and pain without the use of local anesthetics.

M-33. Residual Mercury Content of Amalgam Restorations and Its Influence on Compressive Strength. *M. L. Swartz, B. S., and R. W. Phillips, B.S., School of Dentistry, Indiana University, Indianapolis, Ind.* This investigation was concerned with the general problem of the residual mercury content in the amalgam restoration. A method of mercury analysis was employed which was accurate for use with small and unpulverized samples. This enabled analysis to be made of various areas of amalgam restorations, revealing that the residual mercury content in some portions often approached 60 per cent. An investigation of the effects on compressive strength of residual mercury content in amounts exceeding 50 per cent was undertaken. Such ramifications of the problem as the influence of condensing pressure and the original mercury alloy ratio were also studied. The findings indicate that residual mercury content does vary with the original ratio and with condensation pressure. Although 1 hour and 24-hour compressive strength is not altered appreciably by fluctuations in the residual mercury content between the ranges of 50 per cent and 55 per cent, a serious loss of strength does occur when the mercury exceeds 55 per cent.

M-34. A Proposed Tentative Specification for Chromium-Cobalt Base Dental Alloys. *D. F. Taylor, M.S., A. Adler, B.S., and W. T. Sweeney, B.A. National Bureau of Standards, Washington, D. C.* In recent years chromium-cobalt base alloys have achieved widespread and increasing use in dentistry. A need has been felt for a specification for these alloys corresponding to the American Dental Association and federal specifications for gold alloys. A proposed tentative specification is presented for these materials, based on work done at the National Bureau of Standards and elsewhere. The properties specified include chemical composition, tensile strength, yield strength, elongation, and hardness.

M-35. The Significance of Testing Thermal Properties of Dental Alloys. *K. M. Weigert, Ph.D., Goldsmith Bros. S & R Co.* A recent article by Brumfield (*J. A. D. A.* **49**: 17-30, 1954) expressed the interest of the dental trade in standardizing testing methods for dental materials. These tests have been advocated by the National Bureau of Standards for more than 20 years, and are used throughout the country. This paper takes the technics for granted, and concerns itself only with the meaning of certain tests: (A) The proposed test for the lower limit of the melting range: this was called originally "fusion point" and gave the temperature at which the alloy lost most of its strength before solidification started, being of value for annealing and soldering. (B) The proposed test for the upper limit of the melting range: originally called "flowpoint," this temperature was an indication for the free-flowing temperature of the entire alloy. It will be shown how from the studies of phase diagrams and the interpretation of cooling curves the mechanism of melting and solidification can be established. The temperatures of total solidification (solidus) and total liquidation (liquidus) established from the interpretation of cooling curves might or might not coincide with temperatures determined by other methods, according to the composition of the alloys. Recommendations for casting temperatures have to be empirically determined and mostly they lie considerably above the temperatures that would be considered sufficient ordinarily to liquefy the alloys.

M-36. The Effect of the Contra-Angle Upon the Speed of the High-Speed Handpiece. *James H. Wick, D.D.S., School of Dentistry, University of Iowa, Iowa City, Iowa.* For practical purposes, only standard equipment available on the open market, was used in the tests. Three handpieces with different type bearings designed for operation at high speeds, and their respective contra-angles, were tested at speeds ranging from 8,000 r.p.m. to 16,000 r.p.m. All were uniformly broken in, carefully adjusted, and tested under like conditions. Known variables that would affect the uniformity of the tests were reduced to a minimum. Readings taken simultaneously of the handpiece and motor speeds, by means of 2 tachometers, were used to determine correct belt tension and also to determine the motor speed required to produce a given handpiece speed. With the contra-angle attached and the motor operated at the predetermined speed required to produce the desired handpiece speed, the contra-angle speed was obtained. To reduce error, the procedure was repeated 5 times with each handpiece and contra-angle and the average of the 5 readings was used in computing the comparisons. Results indicated that the differences in the amount of handpiece speed reduction produced by the contra-angles were much greater than the differences in motor speed required by the handpieces to produce a given handpiece speed. Also, the contra-angles required a proportionally greater motor speed to obtain a given bur speed, than did their respective handpiece.

M-37. Recent Ultrasonic Dental Studies, Including Periodontia, Without the Use of an Abrasive. *Doran D. Zinner, D.D.S., Director of Dental Research, The National Children's Cardiac Hospital, Miami, Fla.* Various physical and biologic aspects of the ultrasonic dental method are considered. Thermocouple studies of temperature rise in tooth pulps are presented for teeth cut ultrasonically and with conventional diamond and carbide instruments. There is no appreciable difference in temperature rise between ultrasonics and the diamond with a liquid coolant and no indications of absorption of ultrasonic energy. The effect of the ultrasonic dental tool and cavitation on the hemolysis of blood under controlled conditions is shown, with no change of physical and chemical properties of blood as far as cavitation effects with the intervention of a membrane between the vibrating tool tip and blood. These studies are

related to the physical and biologic aspects. A new potential ultrasonic dental technic in which the instrument vibrating at the rate of 29,000 cycles per second is utilized with water rather than with an abrasive cutting mixture, is described with the results of in vitro and clinical experiments.

V. Abstracts of Papers Read by Title

T1. Gingival Cysts. *S. N. Bhaskar, D.D.S., Ph.D., and D. M. Laskin, D.D.S., M.S., College of Dentistry, University of Illinois, Chicago, Ill.* Cysts of the gingiva are thought to be rare and the cases which have been reported thus far concern microscopic lesions observed in routine study of gingival tissue. The present report concerns 3 clinically and radiologically discernible lesions of the gingiva. All these cysts involved the region between the mandibular canines and premolars, presented as circumscribed, slowly enlarging masses, and in the radiographs appeared as circumscribed radiolucencies. Histologically they were lined by thin, nonhornified, stratified squamous epithelium which was free of ridges. No glandular elements were found in the vicinity of the cysts. It is suggested that these lesions arise from traumatically displaced epithelium.

T2. Evaluation of Resin Film-Forming Materials for Use as Bonding Agents Between Resin-Filling Materials and Tooth Structure. *M. G. Buonocore, M.S., D.M.D., Eastman Dental Dispensary, Rochester, N. Y.* One approach to bonding acrylic-filling materials to teeth is through the use of thin resin films capable of strongly bonding to tooth structure on 1 surface and bonding to the restorative material on the other. Over 100 commercial resin compositions were tested. Water-stored flat dentin surfaces air-dried for 5 minutes to 24 hours prior to film deposition, were used. Films were evaluated on ease of application; resistance to dye pickup and penetration; ability to protect against acid action; resistance to removal by hot and cold water. Satisfactory films were then tested for their ability to bond acrylic-filling material to dentin measuring the perpendicular pull required to separate the acrylic. General failure, due to solubility of films in acrylic mix, indicates that a successful film should possess groups capable of bonding to dentin, yet be insoluble in acrylic. Some films, however, may be useful as cavity linings under silicate or amalgams or in cementation. From all aspects an experimental material (910 adhesive) produced by Tennessee Eastman rated best.

T3. The Rate of Dissolution of the Principal Inorganic Elements of Dentin. *George W. Burnett, D.D.S., Ph.D., Department of Dental Research, Army Medical Service Graduate School, Washington 12, D. C.* The rate of dissolution in Morse's solution of calcium, phosphorus, magnesium, and carbon dioxide (as carbonates) from water-treated, and calcium fluoride- and sodium fluoride-treated dentin was measured at the end of 8 one-hour intervals. The amount of calcium, phosphorus, magnesium, and carbon dioxide (as carbonate) recovered from the decalcifying solutions accounted for 55 to 57 per cent of the total weight lost by the tissues during 8 hours of decalcification. However, the total amount of elements lost was fairly well correlated with the total weight lost, and either is a better indicator of acid solubility than is the determination of the rate of release of any single element or combination of any 2 elements. The ratio between calcium and phosphorus remained relatively constant during decalcification of the different types of dentin, except that treatment of dentin with calcium fluoride resulted in an early increased release of calcium during decalcification. Some variations were found in the phosphorus:magnesium ratio and in the magnesium:carbon dioxide ratio of the decalcifying solutions of the different types of dentin, but it is apparent that fluorides affect the solubility of the apatite moiety rather than differentially influencing the solubility of individual elements within the inorganic molecular structure of dentin.

T4. Carcinogenesis in the Oral Mucosa. *A. P. Chaudhry, D.D.S., M.S.D., School of Dentistry, University of Minnesota, Minneapolis, Minn.* Two hundred twenty hamsters have been divided into groups of 20 animals each. Three different proved hydrocarbon carcinogens, tobacco, betel chew, and snuff, are being used to induce tumors in the pouch mucosa. An attempt is also being made to hasten the production of tumors with repeated applications of 5 per cent croton oil following only 3 applications of chemical carcinogens and 6 months of introducing tobacco, snuff, and betel chew in the hamster's pouch. In the pilot study in 1 group of animals treated with 7-12 dimethyl benzanthracene, we have successfully induced tumors ranging from papilloma to squamous-cell carcinoma with probable metastases. Since monkeys have proved very resistant to experimental induction of carcinoma, an attempt is being made to induce tumors of the oral mucosa and skin with the most potent carcinogens. These carcinogens are being applied 3 times a week. An investigations is being conducted to study the difference in reaction to chemical carcinogens of the oral mucosa as compared to that of skin. Histologic sections are being prepared at regular intervals to study the changes in the mucosa and to compare with that in skin. The changes induced in the hamster by tobacco, snuff, and betel chews are being compared histologically with changes produced in human oral mucosa after prolonged use of snuff and tobacco.

T5. Experimentally Produced Transitory Bacteremias. *Herbert M. Cobe, Ph.D., College of Dentistry, Temple University, Philadelphia, Pa.* Experimental transitory bacteremias were produced by exodontia, operative procedures, and prophylaxis. The role of the collagenous enzymes is mentioned. Immune and nonimmune rabbits show the effects of antibody formations. Some discussion is allotted to the use of antibiotics.

T6. Sodium Fluoride and Sodium Silicofluoride Applied Topically for the Inhibition of New Carious Lesions. *Abram Cohen, D.D.S., and Maury Massler, D.D.S., M.S., Board of Public Education, Philadelphia, Pa., and College of Dentistry, University of Illinois, Chicago, Ill.* Sodium silicofluoride (Na_2SiF_6) 0.9% solution is superior to sodium fluoride (NaF) 4% solution in inhibiting acid-etching of enamel in vitro when applied topically to extracted teeth. These solutions were compared in vivo by applying 1 to the right and the other to the left side of the dental arch in 180 boys, aged 14 to 18 years. A prophylaxis preceded the 1st application. One application was made in 95 boys, aged 16 to 18 years. Two applications were made 1 week apart in 85 boys, 14 to 16 years of age. All were re-examined 1 year later using bite-wing films taken before the 1st application and 1 year later. One hundred fifty-five new lesions were found in the 180 boys on the side treated with Na_2SiF_6 (average, 0.86 new lesion per boy) while 183 new lesions were found on the side treated with NaF (average, 1.0 new lesion per boy). This difference is not significant. No difference was observed between the group given 1 application and that given 2 applications. In each the average number of new lesions on the side treated with Na_2SiF_6 was slightly less than on the side treated with NaF (0.8 v 0.98 and 0.93 v 1.06 new lesion per boy) but these differences are not significant. Why Na_2SiF_6 is greatly superior to NaF in inhibiting acid action on enamel in vitro but does not show the same superiority in inhibiting new carious lesions in vivo, merits further exploration.

T7. The Effect of Large Doses of Ascorbic Acid on Gingival Tissue at Puberty. *M. Michael Cohen, D.M.D., Tufts College Medical School, Tufts College Dental School, and The Boston Floating Hospital, Boston, Mass.* Thirty-five mentally retarded male patients, aged 11-15, at the Myles Standish Camp

in Taunton, were examined carefully and kodachrome photographs and gingival biopsies were taken on 12 of the most cooperative patients. In addition, vitamin C plasma levels were evaluated in the selected cases. The plasma levels ranged from 0.7 mg.% to 2.25 mg.%. Each patient was given 500 mg. of ascorbic acid by mouth for 90 days. No attempt was made to improve oral hygiene measures, which were very poor. The gingival tissues showed marked inflammation and enlargement and in several cases a superimposed acute necrotizing gingivitis. There was no apparent change for approximately 4 weeks. From the 5th week on, however, the inflammation and enlargement of the gingival tissue subsided. At the end of 90 days, all cases showed a marked improvement of the inflamed and enlarged gingiva and a cessation of the superimposed acute necrotizing gingivitis. In addition, the teeth became whiter and there was a resolution of materia alba, calculus, and stain.

T8. Development of the Squamous Mandibular Articulation in the Rat. *J. J. Cunat, D.D.S., M.S., S. N. Bhaskar, D.D.S., Ph.D., and J. P. Weinmann, M.D., College of Dentistry, University of Illinois, Chicago, Ill.* This histologic investigation is based on complete serial frontal sections through the squamosomandibular articulation of 31 normal and 30 *ia* rats ranging from 16 days' insemination age to 30 days after birth. The *ia* rats are a mutation in which bone resorption does not occur and the growth pattern of bone and cartilages can be accurately traced. This study showed that the condylar cartilage differentiates in the body of the mandible at 17 days' insemination and grows backward toward the zygomatic process of the squamosal bone. In the mesenchyme separating the cartilage from the bone a superior and an inferior slit appear at 19 and 20 days' insemination age, respectively. At 21 days' insemination age the anlage of the capsular ligament appears and the differentiation of the squamosomandibular articulation is completed. The articular disc first consists only of fusiform cells, but progressively becomes collagenized. The synovial cavities are lined partly by flattened and partly by cuboidal epithelium. At 15 days after birth villi begin to form and extend into the synovial cavities. The articular fossa is formed by the zygomatic process and the inferior half of the squama of the squamosal bone. It shows rapid bone apposition at its lateral lip and its depth while elsewhere resorption occurs. The direction of the growth of the condylar cartilage is first predominantly backward but later it is upward and outward. The destruction of condylar cartilage and its replacement by bone begins at 19 days insemination age. Modeling resorption of the neck of the condylar process begins at 15 days after birth.

T9. Activation of the Pyruvic Acid Oxidation of the Salivary Microflora. *H. Eggers-Lura, L.D.S., St. Elisabeths Hospital, Holbaek, Denmark.* The oxidation of pyruvic acid by the salivary microflora as measured by the oxygen uptake in a Warburg manometer may be activated by the following substances: cozymase (DPN), adenosine triphosphate (ATP), aneurindiphosphate (thiamin), ascorbic acid, acetic acid, citric acid, and malic acid, cytochrome, and nitrite, while the addition of KCN, CO, and malonate inhibit the respiration. Fluoride does not influence the salivary respiration in a concentration of m/1000.

T10. Electrochemical Estimation of the Physically Dissolved Salivary Oxygen. *H. Eggers-Lura, L.D.S., St. Elisabeths Hospital, Holbaek, Denmark.* In a previous communication the amount of total salivary oxygen (chemically bound plus physically dissolved) has been estimated by the Roughton-Scholander micrograsometric method. By means of a new electrochemical microapparatus (Todt and Teske, *Biochem. Z.* **323**: 192, 1952) the content of dissolved salivary

oxygen could be estimated at 0.18 to 0.25 vol. % ± 0.01 at 25° C. This value corresponds to about half the content in water and is somewhat higher than the mean of the dissolved oxygen content of the blood.

T11. Investigations Into the Aerobic and Anaerobic Degradation of Glucose in Saliva. *H. Eggers-Lura, L.D.S., St. Elisabeths Hospital, Holbaek, Denmark.* Analyses of lactic acid and pyruvic acid after aerobic and anaerobic incubation of glucose and saliva and measuring the oxygen uptake in Warburg manometer showed that the caries resistant salivary types generally form faster and greater quantities of acids than do the caries active ones in the same time. But also the oxidations of these acids will occur at a faster rate in the caries-resistant salivary types. These results could be confirmed by measuring the pH and the E^h of the incubated salivary samples. The caries active salivas showed a slower acid formation and the E^h values generally were lower than those found after incubation of the caries-resistant types (*Deutsch. Za-Mu- und Kieferheilk,* 1954).

T12. Investigations Into the Oxidation-Reduction Potentials of Salivas From Caries-resistant and Caries-susceptible Persons. *H. Eggers-Lura, L.D.S., St. Elisabeths Hospital, Holbaek, Denmark.* By E^h measurements in open vessels with platinum and calomel-electrodes, especially on stimulated and bicarbonate-containing saliva, a stable potential is found which may be used for characterizing the single salivary types, also when the measurements are made at different times of the day. For the caries-resistant salivary group including 50 persons the mean E^h value was + 309 ± 4.7 millivolts and for the caries-active group, also inelding 50 persons, + 237 ± 9.9 millivolts. The differences between the 2 groups are significant. The saliva from the caries-resistant group seems to be saturated with oxygen, as pure oxygen, when passed through the saliva, did not raise the potential to any remarkable degree. The addition of hydrogen peroxide, on the contrary, causes a rapid rise of the salivary potential because of the presence of salivary peroxydase and catalase. Also, substances activating the oxidizing processes of the salivary microflora like cytochrome, adenosine triphosphate, and cozymase will raise the potential. The differences of the potentials of the 2 groups possibly are due to: (1) differences of the content of oxidizing enzymes and biologic oxidation-reduction systems like sulfhydryl systems, coenzymes, vitamins (B_1, B_6, B_2, C) in saliva itself; (2) differences due to the various microflora in the two groups; more aerobic conditions being connected with the metabolism of the flora of the salivas of the caries-resistant subjects.

T13. Investigations Into the Oxygen Uptake of the Salivary Microflora With and Without Added Substrates. *H. Eggers-Lura, L.D.S., St. Elisabeths Hospital, Holbaek, Denmark.* The oxygen uptake of the salivary microflora has been measured in a Warburg manometer under standardized experimental conditions upon the nonstimulated salivas of 50 caries-resistant and 50 caries-susceptible chidlren 5 to 16 years old. The caries resistance was associated with a high salivary respiration rate; the differences between the 2 groups being significant. The addition of different substrates influences the oxidizing processes both by the nature of and the concentration of the substrates. The "natural" carbohydrates, starch and maltose, are more easily oxidized than glucose, sucrose, and raffinose, in the same concentrations (0.2%). The salivas of caries resistant children generally are activated by the substrates in the concentration of 0.2%, while most of the salivas of caries-active children are inhibited in oxygen uptake. An accumulation of the sugar beyond 5% completely stops the microflora respiration.

T14. Paper Chromatography of Adenosintriphosphate in the Salivas of Caries-resistant and Caries-susceptible Subjects. *H. Eggers-Lura, L.D.S., St. Elisabeth's Hospital, Holbaek, Denmark.* The detection and separation of salivary, energy-rich phosphorus compounds is possible by use of the method of Eggleston and Hems (*Biochem. J.* **52**: 156, 1952). Differences in the content of adenosine triphosphate (ATP) and in the saliva of caries-resistant and caries-susceptible subjects could be noted; the former showing greater content than the latter. The presence of ATP in the salivary glands has recently been demonstrated by Fernandes and Junquiera (*Exper. Cell. Res.* **5**: 329, 1953), who found that aerobic glycolysis, phosphocreatine, ATP, and ADP were significantly decreased in nonsecreting glands as compared with normal controls.

T15. The Amino Acid Content of the Proteins of Normal and Fluorosed Rat Incisors. *Elaine S. Feigelman, M.A., and Earl O. Butcher, Ph.D., Graduate School of Arts and Science, New York University, New York, N. Y.* By the use of 2-dimensional paper chromatography of acid hydrolysates of normal and fluorosed rat incisor proteins it has been shown that no significant difference exists between amounts of corresponding amino acids isolated from normal and fluorosed rat incisors or between amounts of total amino acids present. Fluorosis was produced by repeated injections of 2.5% sodium fluoride solution or by use of a 0.053% sodium fluoride solution as drinking water. A fat-free, insoluble protein residue was isolated from whole incisors by dialyzing the powdered teeth against 2% acetic acid followed by 2% hydrochloric acid and extracting the residue with alcohol, then ether. After drying, the protein residue was hydrolysed by autoclaving for 6 hours at 22 psi with concentrated hydrochloric acid and distilled water 1:1. The amino acids were separated by 2 dimensional paper chromatography using phenol and a lutidine reagent. Quantitation was by photometric analysis of the spots developed by 2% ninhydrin solution. The following amino acids were isolated in both the experimental and control groups: phenylalanine plus the leucines, valine, proline, hydroxyproline, alanine, threonine, glycine plus aspargine, glutamic, serine, aspartic, arginine, lysine, cysteic, an unknown, and tyrosine. Traces of glutathione, methionine, and histidine were sometimes found in either the experimental or control samples. These results agree with those reported for the proteins of normal human teeth with the exception of the presence of glutathione.

T16. A Study of Globular and Interglobular Zones of the Dentin and the Fibrillar Matrix Patterns. *Harry E. Frisbie, D.D.S., College of Dentistry, University of California, San Francisco, Calif.* Through the employment of reticulin stains, it has been possible more clearly than by other methods to demonstrate some of the patterns of maturation variability of dentin matrix in the erupted human tooth. Some tooth areas retain globules demarcated by a narrow silver staining line. Dentinal tubules extend through these without change in size of direction, and the matrix staining appears similar to that of the matrix where such zones may not be present. Interglobular areas are likewise bounded by a silver staining line, but staining of the matrix within such zones is well differentiated from that of normal matrix, as well as from predentin. The granular layer of Tomes is regularly demonstrated by this method as a band of these globular zones, but in this area they are closely placed and relatively small. Some small globules extend peripherally into the crown but they are not numerous as in the granular layer of Tomes. In thin sections of dentin, one can observe bundles of fibrils, presumably of collagen, which make up the bulk of the matrix of dentin. Preliminary studies of decalcified teeth have shown these to vary in abundance from place to place in the dentin. Information contributing

to an understanding of some of the variables of maturation of dentin retained in the adult tooth should materially improve understanding of the clinical progress and rate of dental caries.

T17. A Histologic Study of the Effect of Antiformin Upon the Soft Tissue Wall of Periodontal Pockets in Humans. *Irving Glickman, B.S., D.M.D., and Benjamin Patur, D.D.S., Tufts College Dental School, Boston, Mass.* The effect of a 2-minute application of Antiformin upon the soft tissue wall of periodontal pockets in human beings was studied microscopically. Particular attention was directed to the possibility that Antiformin might have a selective effect upon epithelium. In 9 patients 34 periodontal pockets were treated with Antiformin according to the prescribed technic which included neutralization with 10% citric acid. The pockets were then excised and studied in serial sections stained with hematoxylin and eosin and the Hotchkiss leucofuchsin periodic acid method. Periodontal pockets in the immediate vicinity of those treated with Antiformin were resected and examined for comparison. Microscopic examination revealed that in all cases Antiformin acted as a nonspecific necrotizing drug which affected the connective tissue as well as the epithelium. In some instances a segment of the pocket epithelium in the area of attachment was intact, while the remainder of the epitheilal wall and adjacent connective tissue were destroyed.

T18. Further Observations on the Abnormal Black Pigment in the Enamel of the Rat Incisor. *Humberto Granados, Physiological Institute of the University, Basle, Switzerland.* The occurrence of an abnormal black pigment in the rat incisor has been previously reported (*Experientia* **8**: 154, 1952). Further studies have shown the following: (1) The black pigment is not of microbiologic origin since chromogenic bacteria were consistently absent from cultures made in suitable media of material removed from the gingival sulcus and labial surface of incisors with black pigment at different stages of deposition. (2) The animals reared on the stock colony diet (*Experientia* **8**: 154, 1952) are not always completely exempt from black pigment deposition in their incisors, since in these rats a few cases with black pigment have been found, but usually only in small amount. (3) Other experiments, originally planned to study the possible influence of certain dietary factors on the appearance of the black pigment, have indicated that there may exist a seasonal variation in the incidence of this abnormal pigmentation. In the first experiment (*Experientia* **8**: 154, 1952), which was carried out between October, 1951, and January, 1952, from 92% to 96% of the animals which were raised on a purified diet (Groups 1 and 1A) had black pigment. However, in later experiments, it was found that in rats reared on the same diet as that given to groups 1 and 1A, only 15% of the animals raised from March to April, 1952, and none of the rats raised from April to June, 1952, had black pigment. Furthermore, it was observed that the amount of pigment deposited in the incisors of the animals raised from March to April was very small as compared with the amount of pigment deposited in the incisors of the rats that had been reared from October to January. Thus, the incidence and amount of black pigment may be highest in the winter, much lower in the spring, and none during the summer.

T19. Protective Effect of a Vitamin E-Free, Rachitogenic Diet High in Calcium Against the Enamel Depigmentation of Vitamin E-Deficient Rats. *Humberto Granados, Erik Aaes-Jørgensen, and Henrik Dam, Polytechnic Institute, Copenhagen, Denmark.* The incisor depigmentation of vitamin E-deficient rats is influenced by quantitative and qualitative changes of certain nutrients, and can be prevented by various substances different from vitamin E

(*Science* **101**: 250, 1945; **102**: 327, 1945; *Brit. J. Nutrition* **3**: 320, 1949; *Acta pathol. et microbiol. scandinav.* **27**: 304, 1950; *Acta physiol, scandinav.* **21**: 124, 1950; *J. D. Res.* **29**: 689, 1950; **30**: 508, 1951; *Acta pharmac. et toxicol.* **7**: 171, 1951). In order to study the influence of rachitogenic diets on incisor depigmentation the following experiments were made: 40 weanling male rats were distributed into 4 equally large groups and were fed the following purified diets, all of which contained 20% vitamin-free casein, 20% linseed oil, and 5% salt mixture, for a 70-day experimental period: Group 1 (E-deficient, nonrachitic), an E-free diet containing McCollum's Salt Mixture 185 and supplemented with 2.4 A.O.A.C. Chick Units of vitamin D_3 per rat per day; Group 2 (E-deficient, rachitic), an E-free, rachitogenic ration high in calcium (salt mixture: ferric citrate 5 Gm., magnesium sulfate 7 Gm., potassium chloride 8 Gm., sodium chloride 10 Gm., calcium lactate 70 Gm); Group 3 (E-deficient, rachitic), an E-free, rachitogenic diet high in phosphorus (salt mixture: ferric citrate 5 Gm., magnesium sulfate 7 Gm., potassium chloride 8 Gm., sodium chloride 10 Gm., sodium biphosphate 35 Gm., sodium phosphate 35 Gm.); Group 4 (E-sufficient, nonrachitic), an E-rich diet (20 mg. DL, α-tocopherol acetate per 100 grams of diet) containing McCollum's Salt Mixture 185 and supplemented with vitamin D as Group 1. From the 4th week the degrees of enamel depigmentation were recorded (*Odontolog. Tidskrift* **56**: 457, 1948). The results showed that Group 1 and 3 exhibited a high degree of enamel depigmentation (grades 9 and 8, respectively), whereas Groups 2 and 4 exhibited normal incisor pigmentation (grades 0 and 2, respectively). Thus it is concluded that a vitamin E-free, rachitogenic diet high in calcium protects against the enamel depigmentation of vitamin E-deficient rats just as tocopherol.

T20. Bacteriologic Status of the Pulp Chambers and Root Canals of Teeth Requiring Endodontic Treatment. *Louis I. Grossman, D.D.S., D.M.D., School of Dentistry, University of Pennsylvania, Philadelphia, Pa.* The bacteriologic status of 2 separate portions of the pulp cavity, that is, pulp chamber and root canal of teeth requiring endodontic treatment, was studied. A culture was taken of the pulp tissue, pulp remnants, or cavity wall of the pulp chamber depending upon whether a vital pulp, decomposed pulp, or no pulp were present, respectively. Another culture was then taken of the root canal. All cultures were taken prior to endodontic treatment in the school clinic, under the supervision of an instructor. There was agreement between the cultures taken of the pulp chamber and root canal of the same 535 teeth in 79.4% of cases. Of these, 46% were positive and 33% were negative. While negative cultures were secured in 33% of 535 teeth, negative cultures were obtained only in 19% of teeth with necrotic pulps or with areas of rarefaction.

T21. Effect of Sodium N Lauroyl Sarcosinate on the Fall of pH of Tooth Surface Films and Plaques. *John W. Hein, D.M.D., Ph.D., School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Four groups of 5 subjects each were employed on control dentifrice, experimental dentifrice, control nonbrushing mouthwash, and experimental nonbrushing mouthwash. The experiment lasted 3 weeks: 1st week, both brushing groups on control dentifrice, both nonbrushing groups stopped toothbrushing; 2nd week, control brushing used newly labeled control dentifrice, experimental brushing used same dentifrice except for substitution of 0.2% sarcosinate for detergent, control mouthwash used 0.3% NaCl solution containing 0.15% flavor, experimental mouthwash used 0.3% sarcosinate solution containing 0.15% flavor; 3rd week, all groups used initial control dentifrice. Agents were used daily after breakfast and before retiring and, in addition, immediately after noon meal on days of pH determinations which were conducted between 4:30 and 6:30 P.M. The pH of 3

upper anterior tooth areas (brushers—adjacent to interproximal surfaces; nonbrushers—labial surfaces) was determined before and 7, 14, and 21 minutes after a 2-minute 33% glucose rinse on 2 successive days at end of each week. Means and standard deviations of the 30 minimum pH values of each group at the end of the control, experimental, and postexperimental control weeks were: Control brushing group –5.85 ± 0.46, 5.90 ± 0.70, 5.84 ± 0.40; experimental brushing group –5.25 ± 0.31, 5.66 ± 0.49, 5.15 ± 0.35; control mouthwash group –4.63 ± 0.45, 4.88 ± 0.51, 5.65 ± 0.36; experimental mouthwash group –5.12 ± 0.43, 5.62 ± 0.39, 5.75 ± 0.44. In both experimental groups there was a significant decrease in pH fall at end of experimental week.

T22. Effect of a One Millequivalent Potassium Permanganate Solution Upon Experimental Caries in Male and Female Syrian Hamsters. *John W. Hein, D.M.D., Ph.D., and Joel Wisotzky, D.D.S., School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Eleven male and 9 female hamsters approximately 25 days old comprised the experimental group. Ten male and 7 female littermates served as their controls. The diet consisted of the following ingredients: 4% alfalfa, 35% powdered sucrose, 30% whole wheat, 30% whole powdered milk, and 1% NaCl. The experimental groups drank a 1 meq. KM_nO_4 solution ad libitum while the control groups drank distilled water. The animals were sacrificed 86 days from the start of the experiment and their caries scores recorded by the method of Keyes (*J. D. Res.* **23**: 439, 1944). The caries scores may be summarized as follows: The experimental males showed a 9% increase in total caries score over controls; a 14% increase in score of 1st and 2nd molars; and a 3% increase in score of 3rd molars. The experimental females showed a 29% decrease in total caries score; a 43% decrease in score of 1st and 2nd molars; and a 7% increase in score of 3rd molars. These differences in the caries score between experimental and controls in both males and females were not statistically significant. The results are particularly interesting since KM_nO_4 at the same concentration as used in this experiment will inhibit acid production in saliva-glucose mixtures incubated for 3 hours.

T23. The Effect of a 10 PPM Vanadium Drinking Solution on Dental Caries in Male and Female Syrian Hamsters. *John W. Hein, D.M.D., Ph.D., and Joel Wisotzky, D.D.S., School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* A 10 ppm vanadium drinking solution (V_2O_5 dissolved in HCl and brought to pH 6.5) was tested for its effect on caries in 9 male and 10 female hamsters littermated with 11 male and 8 female controls. The animals were 25 to 28 days old at the beginning of the experiment. Drinking solution and diet were supplied ad libitum for 80 days. The results, based upon Keyes' method of scoring caries, were as follows: males—a significant 35% increase in total caries score, a significant 69% increase in 1st and 2nd molars, and a 35% increase in 3rd molars; females—a 33% increase in total caries score, 60% increase in 1st and 2nd molars, and a 14% increase in 3rd molars. These results differ from those reported for V_2O_5 administered in food and by subcutaneous injection (Geyer, *J. D. Res.* **32**: 1953), but since vanadium ion under different pH and oxidation-reduction pressures is capable of assuming positive valancies of 5, 4, 3, and 2 and hence numerous acid, hydroxide, and oxide combinations, it is not considered that these findings are contradictory.

T24. The Effect of Ingestion of Fluoride on Tooth Movement Following Extraction. *Robert Hillblom, D.D.S., M.S., Finn Brudevold, D.D.S., M.S., and Erling Johansen, D.M.D., Ph.D., Eastman Dental Dispensary and School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Sixty

young rats and hamsters were divided into 3 groups, 1 group receiving distilled water, 1 50 ppm and 1 100 ppm of fluoride in the drinking water. The right 2nd maxillary and the left second mandibular molars were extracted after 3 weeks on the fluoride regimen and the animals were sacrificed after 30 weeks. Measurements on enlarged photographs and radiographs of the extent of tooth movement showed no difference in the experimental groups although fluoride was found to be present in bone in concentrations as high as 9,000 ppm. Differences observed in the tooth movement in the rat and the hamster included more proximal but less extrusive and rotational movement in the rat. It was also observed that in the hamsters there was more movement in the mandible than in the maxilla. These findings could be attributed to differences in the dentition of the animals. The rats given 100 ppm of fluoride suffered from loss of weight, severe mottling of the incisors, and slight symptoms of osteoporosis, while the corresponding hamsters appeared to be normal.

T25. Third Report on the Breeding of Caries-immune and Caries-susceptible Strains of Syrian Hamsters. *Harold C. Hodge, Ph.D., Erling Johansen, D.M.D., and John W. Hein, D.M.D., Ph.D., School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* It has previously been reported (*J. D. Res.* **32**: 654, 1953; **33**: 633, 1954) that 2 potentially caries-resistant and 6 potentially caries-susceptible lines of hamsters have been started for inbreeding. These various lines will henceforth be referred to as follows: Resistant—R_1; R_{2a} and R_{2b} (the letters (*a*) and (*b*) indicate that the line consists of 2 sublines originating from 2 pairs of sibs from the same litter). Susceptible—S_1; S_{2a} and S_{2b}; S_3; S_4; S_5; S_{6a} and S_{6b}. A number of litters in the second, third, and fourth generation of inbreeding from all lines except S_{2a} and S_{6b} have been tested for caries susceptibility for proper selection of breeders. The animals were started in the experimental caries-producing regimen at 35 days of age and maintained thereon for 120 to 135 days. The results of these tests are presented below for each line with the number of the animals, sex, mean caries score, and standard deviation: R_1-36 ♂, 49.45 ± 48.3; R_1-34 ♀, 14.7 ± 18.2; R_{2a}-26 ♂, 20.4 ± 34.7; R_{2a}-19 ♀, 10.6 ± 12.0; R_{2b}-30 ♂, 6.9 ± 8.6; R_{2b}-33 ♀, 3.2 ± 3.6; S_1-11 ♂, 85.7 ± 77.2; S_1-15 ♀, 5.5 ± 10.1; S_{2b}-22 ♂, 164.9 ± 37.2; S_{2b}-25 ♀, 125.8 ± 39.5; S_3-35 ♂, 79.0 ± 51.4; S_3-34 ♀, 48.2 ± 35.2; S_4-79 ♂, 33.8 ± 35.4; S_4-47 ♀, 7.8 ± 11.9; S_5-14 ♂, 104.8 ± 74.9; S_5-26 ♀, 56.6 ± 46.1; S_{6a}-37 ♂, 42.2 ± 26.4; S_{6a}-29 ♀, 27.8 ± 21.9. Statistical evaluation of the data shows that the results are highly encouraging for continuation of the project, and, fortunately, suitable animals are available in each line for its propagation.

T26. Early Effects of Filling Materials in the Teeth of Dogs. *Verda Elizabeth James, D.D.S., and Isaac Schour, D.D.S., Ph.D., College of Dentistry, University of Illinois, Chicago, Ill.* Eight different filling materials were placed in cavities prepared by bur and by "airbrasive" in 116 canine teeth of 42 young adult dogs. The survival periods were 1, 2, and 3 weeks. No significant differences were observed following cavities prepared by bur and those prepared by airbrasive. The histologic findings were evaluated in terms of pulpal inflammation (mild, moderate, and severe) and of odontoblastic activity as reflected in the quality and quantity of postoperative dentin. Three types of dentinal responses could be recognized: (*A*) normal; (*B*) increased dentin formation; and (*C*) dentinal hypoplasia. Types *A* and *B* represented a productive and Type *C* a pathologic response. The severity of the inflammatory response in the pulp tended to increase with cavity depth. The filling materials fell into two groups when they were arranged in terms of the inflammatory response. Group *A*, consisting of zinc oxide and eugenol, calcium hydroxide and amalgam, showed only mild inflammation in even deep cavities, and the absence of dentin

hypoplasia. Group *B*, consisting of gutta-percha, acrylic resin, zinc phosphate cement, silicate cement, and gold foil showed a few instances of moderate inflammation even in shallow cavities and a number of instances of moderate or severe inflammation in the deeper cavities. Dentinal hypoplasia occurred in almost all cases of severe inflammation.

T27. Effect of Cavity Preparation Alone on the Human Pulp. *Verda Elizabeth James, D.D.S., and Isaac Schour, D.D.S., Ph.D., College of Dentistry, University of Illinois, Chicago, Ill.* In premolar teeth of 9 young persons, shallow gingival cavities were prepared with a central deeper portion. A thin gold plate was adapted to cover the central portion so that the latter was left unfilled. Zinc phosphate cement was used to fill the remainder of the cavity. This isolated the central cavity from any external irritation except for the burring procedure. Six unoperated teeth from the same patients were used as controls. The teeth were extracted 15 minutes to 28 days later. In the central unfilled portion the response within the 1st 3 hours appeared to be the same as that observed following cavities filled with zinc oxide and eugenol or with gutta-percha. However, a considerable number of odontoblastic nuclei were seen within the tubules of the predentin and the remaining odontoblasts were crowded toward the predentin. In specimens of 2 weeks' survival, granular debris, which apparently represented the fragmented remains of the aspirated odontoblastic nuclei, was seen in the more peripheral portion of the dentinal tubules. The subodontoblastic layer was richly invaded by new capillaries and young cells. Inflammation was minimal. After 4 weeks, the cells in the odontoblastic layer were still reduced in number, but a small amount of partially calcified reparative dentin was evident. Recovery was progressing and inflammation was absent. It appears that the aspiration of the odontoblasts and relatively slow recovery were not related to the cutting procedures per se but to the failure to fill the cavity so that the pressure within the pulp was greater than within the unfilled portion of the cavity.

T28. The Effect of Diets Containing Various Percentages of Sucrose on Initiation and Progression of Hamster Caries. *Erling Johansen, D.M.D., School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Five groups of littermated animals with 5 males and 5 females in each, were started on the experimental regimen at 35 days of age. Group I received the following basal diet: 45% whole wheat flour, 30% whole powdered milk, 14% cornstarch, 5% alfalfa, 2% dried yeast, 2% salt mixture, and 2% liver powder. Groups II, III, IV, and V received diets containing 14, 27, 44, and 59% sucrose, respectively. These formulas were arrived at by first replacing all the cornstarch and subsequently decreasing the amount of whole wheat flour. All animals, except Group V whose diet contained only 10% protein, thrived well. The animals were examined for caries in vivo at 21, 41, 59, 112, 209, and after sacrifice at 270 days. It was found that an increase in sucrose content accelerated the rate of initiation of carious lesions and speeded the progression of established lesions.

T29. A Histochemical Study of Pulpal Calcification. *Percy L. Johnson, B.S., M.S., Ph.D., and Gerrit Bevelander, A.B., M.A., Ph.D., Department of Histology, College of Dentistry, New York University, New York, N. Y.* A comparative study of several histochemical constituents involved in or associated with calification of secondary dentin, denticles, and areas of diffuse calcification in the pulp of mature human teeth was carried out. Examination of normal secondary dentin formation reveals chemical constituents in the ground substance of the pulp and dentin to be similar to those previously

described by us in earlier publications dealing with the histochemistry of developing teeth. In contrast to these findings, calcification which occurs in the pulp proper giving rise to denticles and areas of diffuse calcification do not reveal the presence of phosphatase, glycogen, or nucleic acids in the cells or tissues associated with the formation of this tissue. Acid polysaccharides, mucopolysaccharides, and fibers are, however, prominent constituents of the ground substance of the last 2 types of calcification studied. These substances are also found in the ground substance of other calcified structures so far examined, such as bone and dentin. It appears from these studies that calcification which takes place in the pulp resulting in the elaboration of pulp stones and areas of diffuse calcification can take place in the absence of several chemical components usually associated with the formation of bones and teeth such as glycogen, phosphatase and nucleic acids. These observations further emphasize the probability that polysaccharides are a necessary constituent of the ground substance of calcifying structures.

T30. The Effect of Dihydrotachysterol (A. T. 10) on Collagen Maturation in the Dentin of Rat Incisors. *Viktors Kalnins, M.D., D.D.D., D.M.Sc., L.D.S., College of Dentistry, University of California, San Francisco, Calif. (From the Pharmaceutical State Laboratory, Stockholm, Sweden.)* The effect of A. T. 10 on the dentin was examined in the teeth of 10 rats (initial body weight 81 to 143 grams) and the results were compared with those in the dentin of 6 animals receiving parathyroid hormone. Dosages of 0.3 to 1.0 c.c. per day of oily solution of A. T. 10 (Bayer or Wander) or 1.5 to 3.0 c.c. per day of parathyroid extract (Lilly) were given by mouth, stomach tube, or subcutaneously over a period of 6 to 12 days. Alteration of collagen in dentin was estimated histologically by means of Hansen or azan stains. It was found that the disturbance of collagen during the action of A. T. 10 precedes hypercalcification of dentin. This is in accordance with similar findings by Kalnins and Ledina (*Upsala Läk. Fören. förh.*, Sweden **52**: 235, 1947) in their studies on the effect of parathyroid hormone on the teeth of guinea pigs.

T31. X-ray Ionization Therapy in Dentistry (Preliminary Report on 181 Cases). *Viktors Kalnins, M.D., D.D.D., D.M.Sc., L.D.S., College of Dentistry, University of California, San Francisco, Calif.* This report deals with single and fractionated irradiation of the dental pulp and periodontium in small dosage (15 to 250 air units, "surface doses") in the treatment of spontaneous pains or reaction to heat and cold or percussion.

PULPAL DISCOMFORT IN VITAL TOOTH AFTER:	NO. OF CASES	RESULTS OF X-RAY THERAPY		
		RECOVERY	IMPROVEMENT	NO RESPONSE
Placing amalgam filling or inlay	51	24	12	15
Placing silicate or plastic filling	17	9	3	5
Preparation for veneer crown	4	2	1	1
Reduction of tooth height by grinding	11	10	1	0
Cementum exposure	5	0	3	2
Treatment of exposed pulp with calcium hydroxide	21	15	2	4
Totals:	109	60	22	27

Of the 82 cases in which recovery or improvement is reported, 55 showed prompt response following an initial exposure to the x-ray, whether single dosage (30 cases) or fractionated irradiation (25 cases) was used. Early response is characteristic where benefit is to be derived from this type of therapy. In 28 cases of periodontitis resulting from drugs or trauma in root canal therapy and

filling, recovery was obtained after single (20 cases) or fractionated irradiation. Also, successfully treated were 6 cases of periostitis and osteitis following extraction or injection of local anesthesia; 7 cases with idiopathic pains in the jaws. X-ray therapy in 8 cases of periodontitis, periostitis, and osteomyelitis was combined with administration of antibiotics. In total purulent pulpitis (14 cases) and periapical periodontitis following pulp gangrene (9 cases), x-ray therapy was contraindicated.

T32. Urinary Estrogen in Periodontosis. *Maxwell Karshan, Ph.D., Benjamin Tenenbaum, D.D.S., and Roslyn Friedland, B.S., School of Dental and Oral Surgery, Columbia University, New York, N. Y.* Urinary estrogen determinations were made in 18 female subjects 21 to 37 years of age, in various stages of periodontosis. Twenty-four-hour specimens were collected, generally on the 7th day of the menstrual cycle (1st specimen), and between the 14th and 18th day of the cycle. that is, on the presumed day of ovulation as assessed by the patient from her temperature record (second specimen). The degree of periodontosis was graded as follows: slight–indicating bone loss in specific areas without the necessity of immediate extraction; moderate–necessity of immediate extractions of some teeth, which can be replaced by partial dentures; severe–extraction of all but 6 teeth necessary. Three cases were graded as slight; 8 as moderate; 7 as severe. In all 7 cases classified as severe, estrogen values in the second specimen, ranging from 14.7 to 28.3 μg per 24 hours, were lower than those in the groups classified as slight or moderate. In these latter groups the range of values was from 33.8 to 50.5 μg per 24 hours. However, lower values were not found consistently in the first specimens in the severe periodontosis group. In these first specimens the range was from 11.6 to 32.0 μg per 24 hours for the severe periodontosis group, and 20.4 to 41.6 μg per 24 hours for the combined slight and moderate periodontosis groups.

T33. Arginase Activity of the Salivary Glands and Its Inhibition by Testosterone Propionate. *Charles D. Kochakian, Ph.D., Barbara A. Endahl, and David Hall, Oklahoma Medical Research Institute, Oklahoma City, Okla.* Arginase activity was demonstrated in the salivary glands of the rat and the mouse. The concentration of enzyme activity in the submaxillary and the sublingual glands of the rat was approximately the same. In the mouse, the activity of the submaxillary and sublingual glands was determined together and was approximately 70% of that in the rat. Castration produced a small increase in the arginase activity of the submaxillary and sublingual glands but not the parotid. The difference became somewhat greater as time passed. The subcutaneous implantation of pellets of testosterone propionate (0.37 mg. per day) and methyltestosterone (0.34 mg. per day) for 22 days into castrated rats did not change the weight of the submaxillary and sublingual glands but reduced the arginase activity below that of the normal rats. When 0.5 mg. per day of testosterone propionate was injected for 60 days into castrated rats, these salivary glands were increased in weight and their arginase activity was greatly decreased. Thyroxine at 5 μg and 10 μg per day was ineffective alone or when administered with the androgen. No significant changes were noted in the arginase activity of the parotid glands in any of these experiments. A large number of androgens have been implanted subcutaneously as pellets in castrated mice. After 20 days the combined weights of the submaxillary and sublingual glands were increased as much as twice and the concentration of arginase activity was decreased to less than one-half.

T34. Salivary Acid and Alkaline Phosphatase as Predictors of Oral Conditions. *V. F. Lisanti, D.M.D., and H. H. Chauncey, M.A., Department of*

Dental Research, Tufts College Dental School, Boston, Mass. Acid and alkaline phosphatase activity were determined in the whole saliva of 92 individuals. The population consisted of 5 groups: "normal," dental caries only, periodontal disease only, caries and periodontal disease, and edentulous. Statistical comparison of the groups revealed no significant difference in acid phosphatase activity between the groups except for the edentulous patients. The mean enzyme titer for the edentulous group was significantly higher than those exhibited by the 4 other groups. The confidence levels of differences ranged from 5 to 10%. In a normally distributed population it was not possible to differentiate oral condition, except for the edentulous individuals. Only when more extreme values were included (both high and low) was it possible to differentiate caries free from rampant caries cases 36% of the time. Although the acid and alkaline phosphatase values had a correlation r equal to 0.55 with a level of confidence of 1%, the alkaline phosphatase mean values did not differ significantly among any of the 5 groups.

T35. The Effect of Unbalance of Adrenal Hormones on Pulp Reactions and Dentin Deposition. *Vincent F. Lisanti, D.D.S., and Rachel G. Hill, B.S., Tufts College Dental School, Boston, Mass.* The dogs were pretreated for 1 to 4 weeks with doses of 1.5 mg. per kilogram of either cortisone or DCA (desoxycorticosterone acetate) and compared with the responses of the untreated dogs for similar periods of time. Cavity preparations with a dental bur and the "airdent" technics were compared. One of the injuries used was heat applied to the floor of the cavity preparation, varying from 200° F. to 600° F., followed by a zinc oxide and eugenol restoration. Cavity preparations without heat injury were restored with zinc oxide and eugenol, silicate cement, zinc phosphate cement, methyl methacrylate resins, and no restoration. Pretreatment for longer than 2 weeks demonstrated the following: (1) interference with macrophage activity; (2) differentiation of the pulpal stellate mesenchymal cells to other cell types (fibroblasts and odontoblasts) related to pulpal healing was retarded; (3) increased blood vessel fragility and a reduction in the numbers of leukocytes especially eosinophils and lymphocytes; (4) there appeared to be an increase in the number of plasma cells; (5) with cortisone there was a reduction in the number of young fibroblasts and to a lesser degree the ground substance between cells; (6) with DCA there was an increase in young fibroblasts, fibrils, and ground substance; (7) both demonstrated almost a complete absence of predentin formation and no calcification was seen in any of the hormone pretreated animals; (8) when cortisone or DCA was administered postoperatively there was a general delay in healing or repair of the dental pulp; (9) the dogs treated with cortisone 2 months postoperatively showed a slightly better reparative response than with DCA although considerably less than the untreated animals; (10) the cortisone-treated animals' dental pulps were indistinguishable from the untreated control; (11) the DCA-treated animals, however, showed increased vascularity, a dramatic increase in the number of young stellate mesenchymal and fibroblastic cells, with an accompanying increase in fibrils and ground substance.

T36. Desensitization of Cervical Cementum and Dentin by Sodium Silicofluoride. *Maury Massler, D.D.S., M.S., College of Dentistry, University of Illinois, Chicago, Ill.* Sodium silicofluoride 0.9 per cent aqueous solution, was compared with sodium fluoride 2 per cent, formalin 10 per cent, zinc chloride 10 per cent, and silver nitrate 10 per cent solutions for the desensitization of sensitive necks of teeth. Twenty-one cases with painful cervical erosions were investigated by applying 1 of the solutions to the teeth on 1 side, and the sodium silicofluoride solution to similar teeth on the other side for 5 minutes each. The

patients invariably reported greater desensitization in the teeth treated with sodium silicofluoride. A similar experiment was conducted by 4 periodontists who applied the solutions to sensitive cementum following gingivectomy. To date 40 case histories indicate a definite superiority of sodium silicofluoride over the other substances used to desensitize surgically exposed cementum and dentin. The mechanism of this action is not known.

T37. Caries Resistance in the Hamster. *H. David F. Mitchell, D.D.S., Ph.D., and Marilyn Johnson, B.S., School of Dentistry, University of Minnesota, Minneapolis, Minn.* Inability to induce appreciable amounts of occlusal caries in hamsters in recent years has been reported from several laboratories as well as our own (*J. D. Res.* **33**: 676, 1954). Another effort was made as follows: 42 ♂ and 44 ♀ were fed a fortified breeding diet high in sugar pre- and postnatally until the age of 12 days. Then 24 ♂ and 25 ♀ (experimental) were placed on a previously tested cariogenic diet and maintained for 100 days. The remaining animals (controls) were fed laboratory chow from 12 until 30 days of age, and then placed on the same "cariogenic" diet for 100 days. Two surveys, in vivo, revealed practically no gross caries after 100 days, so only representative numbers of each group were sacrificed. Microscopic study of the jaws showed: Controls, 2♂, 1 cavity; 7♀, 1 cavity; 7 animals caries-free: Experimentals, 10♂, 10 cavities; 10♀, 7 cavities; 11 animals caries-free. The cavities detected were uniformly small. Food and water F content was very low except for the chow. Six highly inbred young Chinese hamsters from Boston University developed no caries after 100 days of a "cariogenic" diet. Five albino hamsters from another colony developed considerable caries. With the exception of the albinos, this failure to induce the degree of caries which once was so common in our laboratory under much less stringent circumstances again seems to point up a difference in susceptibility due to strain differences or some other unexplained factor.

T38. The Significance of the State of the Fluoride in Drinking Water. *D. Morken, Ph.D., I. Feldman, Ph.D., and Harold C. Hodge, Ph.D., School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* With the aid of a Beckman pH meter, Model G, pH measurements were made on successive dilutions of a 10^{-2} molar aqueous solution of sodium fluorosilicate at room temperature, 29° C. Assuming $pH \cong -\log_{10}$ (hydrogen-ion activity) and making use of Rees and Hudleston's calculated value of 1×10^{-6} for the equilibrium constant of the reaction

$$SiF^{=}_{6} \rightleftarrows SiF_4 + 2F^-$$

a hydrolysis constant of 10^{-31} was determined for the reaction

$$SiF^{=}_{6} + 3H_2O \rightleftarrows H_2SiO_3 + 4H^+ + 6F^-.$$

This hydrolysis constant may be used to obtain information concerning the state of fluoride in drinking water. Calculations show that at the total fluoride concentration prevailing in drinking water ($\sim$ 1 ppm for artificially fluoridated water and 10 ppm or less for naturally fluoridated water) sodium fluorosilicate is completely hydrolyzed at pH 5 and above. At pH 5, and above, the fluoride content in fluoridated water consists of fluoride ion, hydrogen fluoride, and fluoride complexed by metallic ions (such as iron, aluminum, calcium, and magnesium). At pH 5, the hydrogen-fluoride concentration is about 1.5 per cent of the fluoride-ion concentration. At pH 7, and above, the hydrogen-fluoride concentration is negligible. The presence of metal ions which are complexed by fluoride ions would aid the hydrolysis of fluorosilicates. The stability constants of the monofluoro complexes of metals may be employed to approximate the complexed fluoride concentration. For instance, using the constants reported

by Connick and Tsao, it was calculated that when drinking water in the Rochester, New York area is fluoridated to an extent of 1 ppm total fluoride, a maximum of 20 per cent of the fluoride would be complexed to iron, calcium, and magnesium, and the remaining 80 per cent would be fluoride ion.

T39. A Procedure and Technique for Studying the Local Effect of Anterior Hypophyseal Growth Hormone Upon the Growth of the Guinea Pig Mandible. *Hugh I. Myers, Ph.D., and Kirk C. Hoerman, D.D.S., M.S., School of Dentistry, University of Kansas City, Kansas City, Mo., and U. S. Naval Dental Research Facility, Great Lakes, Ill.* Fifteen μg of a purified growth-promoting bovine pituitary extract (Armour Laboratories, Lot No. K45408-R) was injected within the confines of the capsular ligament of the right mandibular joints of 4 normal male 9 week-old guinea pigs every 48 hours for a period of 2 weeks. The right and left joints were removed, decalcified, embedded in paraffin, sectioned serially, and stained (every 10th). The rate of growth was calibrated by counting the number of osteoclasts present about the condyle head. Earlier, Myers and Reeve established average osteoclast counts for normal male guinea pig joints ranging in age from 2 to 52 weeks. Average osteoclast counts for 9-week-old animals were 1,198 cells per joint. The average cell counts for the 4 treated right joints were 1,206 cells per joint. The osteoclast counts of the 4 treated joints were 17 per cent greater than the cell counts of the 4 opposite untreated joints. The greatest single increase was 29 per cent. This increase resulted by a decrease in count from the normal in the left untreated joints. None of the 8 mandibular joints studied showed changes in the zones of osteogenic activity which could not otherwise be attributed to normal variation. The data are not sufficient to show a local effect of growth hormone upon the growth of the guinea pig mandible. There is, however, an indication for further study and refinement of technic.

T40. Eruptive Movement of Lower First Molar of the Rat. *C. O'Brien, D.D.S., M.S., S. N. Bhaskar, D.D.S., Ph.D., and J. P. Weinmann, M.D., College of Dentistry, University of Illinois, Chicago, Ill.* This histologic study is based on serial sections through the first molar region of 64 rats ranging from 13 days' insemination age to 30 days after birth. Twenty of the animals belonged to the *ia* strain which is characterized by absence of bone resorption. Thus the mandibles of these rats show various fixed points which permit accurate comparisons of successive developmental stages. A comparison of *ia* and normal littermates revealed the role of bone resorption. This study showed that the movement of the first molar begins with its initiation and continues during the entire period studied. Prior to root formation the upward and outward movement of the tooth germ keeps abreast with the corresponding growth of the alveolar process. Formation of the root, rapid localized bone apposition in the crypt, and localized proliferation in the follicle are associated with the appearance of the tooth in the oral cavity and its outward tilt in the eruptive process. The connective tissue of the follicle is at first loosely arranged, but, with age, successively changes to highly cellular, collagenized, and highly collagenized tissue. The follicle is always wider on the side from which the tooth germ migrates. This investigation shows quantitatively that the eruption is a continuous process which begins with initiation and depends upon the growth of the dental pulp, dental follicle, and the periodontal membrane.

T41. Effects of Diaphragms on the Intensity of Radiation in Air. *Eugene E. Petersen, D.D.S., M.S.D., School of Dentistry, University of Minnesota, Minneapolis, Minn.* Three diaphragms, which could be varied in size from 3 inches to ½ inch, were constructed and placed in a line between the target

of the dental x-ray machine and an ionization chamber. One set of readings was made having 18 inches between the diaphragms. A 2nd set of readings was made having 9 inches between the diaphragms. In each set of readings the size of the diaphragms was varied between ½ inch and 3 inches. Thirty readings were made for each of the diaphragm settings, of which there were 16. The 1st series of readings in which the diaphragms were 9 inches apart and the diaphragm sizes varied between ½ inch and 3 inches, the readings varied from 88.4 milliroentgens for the large diaphragms and 96.0 milliroentgens with no diaphragm. In the 2nd series of readings in which the diaphragms were 18 inches apart the readings were corrected for the inverse square law and found to vary from 84.4 milliroentgens for the small diaphragms to 92.4 milliroentgens with the large diaphragms and 97.6 milliroentgens with no diaphragm. It was found that, in general, as the size of the diaphragm became smaller, the intensity of the radiation became smaller. It was further noted that as small diaphragms were placed closer to the ionization chamber there was a tendency to slightly increase the intensity of the radiation. The 1st result was from the reduction of the field of radiation while the 2nd result was from secondary radiation from the edges of the diaphragms.

T42. Defect Apatite Series. *Aaron S. Posner, Ph.D., American Dental Association Research Fellowship, National Bureau of Standards, Washington, D. C.* A series of calcium phosphates has been prepared which vary in chemical analysis but all of which give the x-ray diffraction pattern of hydroxyapatite. This is similar to the calcium phosphate phase of bone and tooth mineral where there is an apatite phase varying in chemical composition among specimens. A defect series in the hydroxyapatite family is suggested to explain this condition. By postulating a statistical absence of calcium ions in the hydroxyapatite structure one can explain the drop in index of refraction with the drop in Ca/P ratio of these compounds while their x-ray patterns remain the same. This theory also explains the uptake of calcium ions from solution by these various defect apatites. The compound described by Dallemagne, Brasseur, and Melon, alpha tricalcium phosphate, now becomes a specific member of this series with a Ca/P ratio of 1.95. One of the tests used to discriminate alpha tricalcium phosphate from pure hydroxyapatite is calcination at 900° C., whereupon the alpha changes to a "beta" phase and the hydroxyapatite remains the same. All of the defect apatites, prepared above, give a "beta" x-ray diffraction pattern after the same calcination at 900° C. This indicates that this high temperature phase change is a property of the entire defect series and not of alpha tricalcium phosphate alone. We must now regard the "beta" tricalcium phosphates as another defect series with more and less calcium missing from the ideal "beta" structure. Proof is offered to show that the variations in the Ca/P ratio of the defect hydroxyapatite series is not due to the surface adsorption of phosphate ions.

T43. Carbohydrates of Human Saliva. *Jane Reid, M.S., William Hawkins, M.S., and Ward Pigman, Ph.D., Medical College and School of Dentistry, University of Alabama, Birmingham, Ala.* The possible presence of glucose in saliva has been investigated by a number of different methods. Secretions of the 3 large paired salivary glands were collected separately and analyzed. Ethanol extracts of lyophilized saliva and salivary gland secretions were examined by paper chromatography. No evidence was found for the presence of any glucose or other simple aldoses or ketoses. If present at all, their concentration must be less than about 5 mg. per 100 c.c. These results were confirmed by reducing power determinations of the ethanol extracts and of deproteinized saliva. All the salivary secretions have a reducing power, which has usually

been ascribed to the presence of sugars. The reducing power differs considerably for the individual secretions, but is shown by all. The secretions of the submaxillary and parotid glands, which are usually considered the main source of human saliva, have a much lower reducing power than whole saliva. The secretion from the sublingual and accessory glands exhibits much reducing power and probably accounts for a large portion of the reducing power of whole saliva. The reducing materials in whole saliva appear to be composed of a mixture of materials of diverse nature and origin. Mucin may account for some of the reducing power particularly in the submaxillary and sublingual secretions. The other reducing materials are unknown.

T44. Achromycin and Hydrocortisone in Combination in Treatment of Oral Conditions. *Lester Hugh Roth, D.D.S., School of Dentistry, University of Pittsburgh, Pittsburgh, Pa.* Preparations of tetracycline with hydrocortisone, free alcohol, and hydrocortisone acetate were used in various oral conditions. Patients previously experiencing local or gastrointestinal sensitivity with the antibiotics, oxytetracycline, chlortetracycline, tetracycline, or varied preparations of penicillin, were given the new preparations. Two preparations were tested. The initial preparation containing achromycin-HCl and hydrocortisone free alcohol caused nausea and vomiting; however, no local side reactions in the oral cavity occurred. A 2nd preparation of achromycin-HCl with hydrocortisone acetate resulted in lessening of nausea, vomiting, and other side effects. It may be concluded from these trials that the preparation containing tetracycline with the hydrocortisone acetate is efficacious in oral treatment.

T45. Dental Effects of Dietary Aureomycin. *Martin A. Rushton, L.D.S., M.D., Guy's Hospital, University of London, London, England.* Dietary Aureomycin has been shown to affect the growth rates of certain animals; by altering the oral flora it might be thought also to affect the progress and pattern of periodontal disease. Three groups of 10 female hamsters were fed from weaning for 8 months on diets containing 35 per cent milk powder, 28 per cent, and 28 per cent plus 0.002 per cent Aureomycin. The mean final weight differed little in the 3 groups but was slightly less in those having Aureomycin. Serial sections showed the general pattern of downgrowth of the epithelial attachment on the molar roots of 6 animals to be the same in all groups, but to be significantly retarded in the Aureomycin group as compared with both the others. It is concluded that the addition of Aureomycin was without effect on the pathologic process leading to the formation of deep periodontal pockets, but delayed passive eruption of all the molars. The thickness of the dentin of the lower incisors was significantly greater in the low-milk plus Aureomycin group than in either of the other groups and the maturation of enamel significantly more advanced. In some animals of the low-milk group the dentin was so thin that the pulps were exposed by attrition although the teeth were not shortened.

T46. The Salivary pH of Rodents. *C. A. Schneyer, Ph.D., and J. F. Volker, D.D.S., Ph.D., School of Dentistry, University of Alabama, Birmingham, Ala.* Attempts to modify experimental dental caries in rodents with selected therapeutic agents have given results that are at variance with those observed in clinical studies with human beings. These differences suggest the possibility that the normal oral environment of rodents may not be analogous to that in human beings. Accordingly, we have studied the salivary pH of hamsters, rats, and mice. The following methods have been used: (1) The insertion of indicator papers into the oral cavity and the notation of their pH changes. (2) The introduction of cellulose sponges into the mouth and determination of the pH of the absorbed saliva with a one-drop glass electrode (Beckman pH meter).

(3) The direct collection of saliva after subcutaneous injection of 2 mg. of pilocarpine and the determination of its pH with a one-drop glass electrode (Beckman pH meter). The salivary pH of the rodent groups was found to vary from 8 to slightly more than 9. The pH of hamster saliva tended to approach the latter figure, that of mouse saliva the lower value. Human saliva tested under similar circumstances gave readings between pH 6 and 7. A literature survey indicates that salivary pH of ruminants is comparable with that of rodents. These findings may be of significance in interpreting the results of experimental caries studies in rodents.

T47. The Effect of the Systemic Administration of Estrogenic Hormone Upon the Alveolar Bone of White Mice. *Gerald Shklar, D.D.S., and Irving Glickman, D.M.D., Tufts College Dental School, Boston, Mass.* The effect of weekly subcutaneous injections of 600 rat units of alpha estradiol benzoate (Progynon B. Schering) upon alveolar bone was studied in young adult white mice approximately 6 weeks old, weighing from 18 to 24 grams. Eighty animals were used for experimental periods of 5 and 10 weeks. Uninjected animals and animals receiving weekly injections of sesame oil served as controls. No differences were observed between the estrogen-injected and the control animals with respect to size, weight, or rate of tooth eruption. Microscopically, the interdental bony septa of the 5-week estrogen-injected animals were similar to the controls. In the 10-week estrogen-injected animals there was little or no evidence of bone apposition normally found adjacent to the periodontal membrane. In both the 5- and 10-week estrogen-injected animals the interradicular bony septa differed from the controls in that the central portion in relation to the marrow spaces occupied a disproportionately large area and was sparsely cellular. There was a slight suggestion of the normal circumferential lamellar arrangement of bone immediately surrounding the marrow spaces. However, for the most part, the bone in the central portion of the interradicular septa was coarsely fibrillar and devoid of normal incremental and reversal lines. The prominence of relatively immature bone is suggestive of increased endosteal bone formation resulting from systemically administered estrogen.

T48. Azocoll as a Substrate for Determining Proteolytic Activity. *Leo M. Sreebny, D.D.S., Ph.D., Julia Meyer, B.S., and Erica Bachem, B.S., College of Dentistry, University of Illinois, Chicago, Ill.* Azo dyes coupled to the histidine and tyrosine groups in proteins have been used as substrates in testing for proteolytic activity. Hide-powder, when coupled with diazotized benzidine, gives a reddish purple indicator substance to which the name azocoll was given. The present study was undertaken to explore whether azocoll was suitable as a substrate for determining proteolytic activity. The results show that: (1) azocoll is decomposed by trypsin, collagenase, papain, chymotrypsin, and pepsin. It is not attacked by hyaluronidase, streptokinase, streptodornase, and beta-amylase; (2) there is no spontaneous decomposition of azocoll within the pH range of 1.0 to 12.2 or within the temperature range of 37 to 70° C. during a period of 40 minutes; (3) maximal light absorption by the dye fraction of decomposed azocoll occurs at 522 mμ; (4) the dye released by the decomposition of azocoll remains virtually stable at room temperature for over 4 hours; (5) duplicate determinations of enzymatic decomposition of azocoll agree within a maximal error of 6 per cent for concentrations not exceeding 2 mg. per millimeter of incubating mixture. The use of azocoll is limited chiefly by the difficulty of dissolving it in high concentrations and by the lack of uniformity of different preparations. On the other hand, its stability under a wide range of conditions makes it easy to use, and it lends itself to rapid and fairly accurate determinations.

T49. The Organic Elements of Enamel. VI. A Comparison of the Histomorphology of Enamel as Observed in Ground, Decalcified, and Plastic-Embedded Thin Sections. *R. F. Sognnaes, L.D.S., Ph.D., and J. T. Albright, D.D.S., School of Dental Medicine, Harvard University, Boston, Mass.* This study summarizes and correlates the microscopic appearance of enamel as observed in ground sections, decalcified paraffin sections, and in thin sections of plastic-embedded specimens. It appears that the generalization may be made that the histomorphologic characteristics of enamel can be largely explained on the basis of the pattern of its organic framework. The fibrous nature of this framework is demonstrated by polarized light microscopy and details of the intraprismatic structure are revealed by examination of thin sections by dark-field microscopy.

T50. Oral Health Conditions Among Army Personnel at the Army Engineer Center, Fort Belvoir, Va. *S. S. Stahl, D.D.S., M.S., and A. L. Morris, D.D.S., Dental Clinic, U. S. Army Hospital, Fort Belvoir, Va.* Eleven hundred fifty-three white male army personnel, 373 officers and 780 enlisted men, ranging in age from 17 to 49 years, and having 6 or more natural teeth, were examined by means of tongue depressors and artificial light for evidence of changes in gingival appearance, gingival recession, tooth loss, and presence of white chronic lesions of the oral mucosa. Gingival changes were recorded using a modification of the P-M-A Index. The number of inflamed gingival papillae increased with aging from an average of 35 per cent of papillae involved in ages 17 to 19 years to 43 per cent in ages 40 to 49 years. The percentage of marginal gingival inflammation and gingival recession also increased with aging. Statistical analysis showed the officer group to have significantly lower papillary involvement at ages 30 to 49 years, lower marginal involvement at ages 20 to 49 years, and lower gingival recession at ages 20 to 39 years than the enlisted group. Tooth loss increased with aging from an average of 6.0 teeth at 17 to 19 years to 8.0 teeth at 40 to 49 years and was significantly lower at all age levels in the officer group. Oral health conditions were found to be similar in soldiers from different geographic locations. The oral health conditions of 150 Negroes, 17 officers and 133 enlisted men, ranging in age from 20 to 39 years, examined by the foregoing methods, were found to be similar to those seen in the white group of comparable age. Of the white group, 5.2 per cent showed evidence of chronic white lesions of the oral mucosa.

T51. Effect of Methylandrostenediol on Periodontosis. *Benjamin Tenenbaum, D.D.S., Roslyn Wiener, M.D., Henry I. Nahoum, D.D.S., and Maxwell Karshan, Ph.D., School of Dental and Oral Surgery, Columbia University, New York, N. Y.* An earlier study of urinary neutral 17-ketosteroid excretion, as an index of androgen production, in 25 female subjects with periodontosis, revealed a distribution of values within the normal range, with a mean for the group that did not differ significantly from that of a group of females of comparable age who were free from periodontal disease. However, in view of the striking benefits observed in postmenopausal and senile osteoporosis after the administration of androgens and estrogens, it was decided to try hormone treatment in our patients. We sought to avoid the menstrual disturbances frequently associated with estrogen therapy, and the masculinizing effects of testosterone administration. The steroid methylandrostenediol has been found to be effective in an anabolic capacity without the production of masculinizing changes. Hence we decided to employ this compound in a group of patients with periodontosis of various degrees of severity. Fourteen patients were studied. Each subject was given 50 mg. of methylandrostenediol per day orally, and serial dental examinations and dental x-rays were made at 3-month intervals for a period of 6 months

to 1 year. The patients, in general, exhibited increased appetite and gained weight, suggesting an anabolic stimulus. On the basis of radiographic examinations involving observations of the periodontal membrane, contour of the alveolar crest, and presence of the lamina dura at the crest, no appreciable change was noted between the condition at the initial examination and that after completion of administration of the hormone.

T52. The Effects of Solutions of Cadmium Sulfate and Lead Acetate on the Incisor Pigmentation, Fur Color, and Blood in Male and Female Syrian Hamsters. *Joel Wisotzky, D.D.S., and John W. Hein, D.M.D., Ph.D., School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* During the course of hamster caries experiments it was noted that there was bleaching of the furs of the male and female hamsters drinking ½ meq. Cd^{++} in the form of $CdSO_4$. The incisor teeth were also uniformly bleached in these same animals. In comparison, animals on ½ meq. Pb^{++} in the form of $Pb(C_2H_3O_2)_2$ maintained good fur color and demonstrated no obvious incisor bleaching. At about the mid-point of the experiment the toxicity of Cd required the concentration to be reduced to ¼ meq. Even at ¼ meq., which is approximately 14 ppm of Cd, the bleaching still continued. After 86 days on the solutions, the animals were sacrificed and Kodachrome transparencies taken of the fur and teeth. Blood analyses were made on the first blood drops following decapitation. In the 8 cadmium males, the average red blood cell count was 7.04 million with an average hemoglobin value of 10.25 Gm. per 100 ml. The male control had an average red blood cell count of 6.28 million and an average hemoglobin value of 16.13. In the six cadmium females, the average red blood cell count was 6.58 million with an average hemoglobin value of 8.33. The average female control had a red blood cell count of 6.77 million and an average hemoglobin value of 15.08 Gm. The Pb^{++} animals showed no variation in color, counts, or hemoglobin from their controls.

T53. The Effect of Sodium Lauroyl Sarcosinate (SLS), Sodium Lauryl Sulfate ($SLSO_4$), and Dehydroacetic Acid (DHA) on Occlusal and Smooth Surface Caries in the Rat. *I. Zipkin, Ph.D., and F. J. McClure, Ph.D., National Institute of Dental Research, National Institutes of Health, Public Health Service, U. S. Department of Health, Education and Welfare, Bethesda, Md.* The present investigation utilized Diets 585 and 636 which, respectively, produce occlusal and smooth surface caries almost exclusively. The data are presented below.

TREATMENT	RATS NO.	RATS WITH CARIES %	CARIOUS TEETH PER RAT NO.	CARIOUS AREAS PER RAT NO.	CARIES SCORE	CARIES SCORE PER CARIOUS RAT
585	30	93.3	3.6	8.2	20.4	21.8
585 0.5% SLS	28	89.3	2.8	4.1	9.4	10.5
585 0.5% DHA	30*	—	—	—	—	—
585	15	100.0	3.5	7.2	16.7	16.7
585 0.1% DHA	15	93.3	3.6	6.7	14.9	16.0
585 0.3% DHA	15*	—	—	—	—	—
585 0.5% DHA	15*	—	—	—	—	—
585	30	100.0	3.6	7.6	18.0	18.0
585 0.5% SLS	30	86.7	2.4	3.6	6.9	8.0
585 0.5% $SLSO_4$	28	92.9	3.1	7.1	16.1	17.4
636	36	69.4	1.5	2.1	2.3	3.3
636 0.5% SLS	35	31.4	0.6	1.0	1.0	3.2
636 0.1% DHA	28	100.0	7.1	16.5	30.5	30.5

*All rats died within 3 weeks.

Sodium lauryl sulfate, although more surface active than SLS, was ineffective in reducing either the incidence or severity of caries. It appears unlikely, therefore, that the cariostatic effect of SLS against occlusal caries is associated with its detergent properties. SLS had no effect on diet intake or weight gain, whereas DHA caused a significant reduction in both. It appears that the effects of SLS and DHA on occlusal surface caries are different from those on smooth surface caries. These data suggest the possibility that the etiology of these two types of rat caries may differ.

T54. Lactic Acid Formation by Lactobacilli Exposed to Fluoridized Enamel. *Jack D. Zwemer, D.D.S., Ph.D., School of Medicine, College of Medical Evangelists, Loma Linda, Calif.* The inhibition of acid production by oral microorganisms exposed to fluoridized enamel was investigated in vivo as a possible factor in the control of dental caries. The intact upper first incisors of 14 young adult human men were isolated and cleaned according to clinical procedures. Unbuffered sodium fluoride (4 per cent w/v) was applied to 1 incisor of each subject for 20 minutes. Sodium chloride (5.56 per cent w/v) was placed on the other incisor as a control. Eight microliters (0.008 ml.) of a 25 per cent washed cell suspension of *Lactobacillus casei* #4646 in a 0.2 M acetate buffer (pH 4.5) containing 1 M glucose was dispensed from a microburette to the labial surface of each tooth and confined within a metal cylinder previously luted to the enamel. In 6 subjects the suspensions were placed on the fluoridized teeth immediately after treatment and water rinsing; in 4 subjects, 24 hours after treatment; and in 4 subjects, 7 days after treatment. All cell suspensions were in contact with enamel for exactly 30 minutes. Aliquots of each suspension were then analyzed for the lactic acid derived from bacterial degradation of glucose by a modification of the method of Barker and Summerson. An average 56 per cent inhibition of lactic acid formation was demonstrated in suspensions confined to the fluoridized teeth immediately after treatment. An inhibition of 14 per cent was observed in suspensions confined to the fluoridized teeth following a 24-hour exposure to saliva. No inhibition of acid formation could be demonstrated in suspensions confined to the fluoridized teeth after 7 days. It may be concluded that the clinical efficacy of topical fluorides in reducing the incidence of dental caries is probably not associated with a significant inhibition of bacterial glycolytic enzyme activity.

VI. Executive Proceedings

A. Council Activities

During the year between meetings the Council nominated candidates for the general offices of the Association constituting the official ballot. A Council committee (Williams, Sognnaes, McClure) reviewed the credentials of persons nominated to membership during the year.

Council Meetings, March 18, 19, and 20, 1955

A quorum was present at all meetings. After discussion of the monetary situation in countries outside North America the dues for the 1956 year were assessed as follows:

Members of the North American Division, $10.00, out of which $3.00 was appropriated to the general fund of the Association and $7.00 for subscriptions to the Journal of Dental Research.

Members residing outside North America, $3.00 dues. The fee for subscription to the Journal of Dental Research, $7.00, is payable only if the member wishes to receive the Journal.

The officers are authorized to reach a compromise on dues for any individual residing outside North America.

The committee on meeting places was directed to select meeting places for 6 years in advance. Dr. Hill, Chairman of the Committee on Incorporation, reported that incorporation of the Association was desirable. The committee was instructed to investigate further and to proceed with incorporation at a suitable time and in whatever jurisdiction is most advantageous to the Association. Dr. Blayney, Chairman of the Committee on Reorganization, (Blayney, Dean, Burrill) submitted a draft of a proposed revision of the constitution and by-laws of the Association. The council either approved or gave detailed instructions concerning several parts of the revision, and instructed the committee to continue its labors.

B. EXECUTIVE SESSIONS

First Session, March 18, 1955, 9:00 A.M.

The meeting was opened by President Paffenbarger with appropriate remarks. Reports of committees and officers were approved and accepted, as follows:

Dr. H. B. G. Robinson, concerning the JOURNAL OF DENTAL RESEARCH. At his request a motion was passed approving use of funds from the Supporting Associates to provide extra pages in the JOURNAL. Dr. Frank Hall for the Publication Committee. Dr. Paul Kitchin for the Novice Award Committee. Dr. George Paffenbarger for the Committee on Members and Divisions Outside North America. Dr. George Paffenbarger for the Program Committee. Dr. J. Volker for the Committee on Selection of Future Meeting Places. Dr. D. Burrill, Assistant Secretary-treasurer on finances.

Tellers were appointed to count the votes for general officers of the Association and to report at the final Executive Session.

After expressions of thanks and appreciation to the several committees and reading of a greeting from the Federation Dentaire Internationale, the meeting was adjourned until March 20, 1955, at 5:00 P.M.

Final Session, March 20, 1955, 5:00 P.M.

The minutes of the Thirty-second annual meeting of the Association, as printed in the JOURNAL OF DENTAL RESEARCH (33: 720-728, 1954), including the report of expenditures therein, were approved.

Reports of expenditures in the conduct of the offices of Secretary-treasurer, Assistant Secretary-treasurer, and Business Manager of the JOURNAL were accepted and approved. Budgets for the operation of the same offices and for the Publication Committee were presented and approved. The officers were authorized to expend up to $1,000.00 from the general funds of the Association for extra pages in the JOURNAL, provided the money is available and the expenditure can be made without jeopardizing the financial condition of the Association. The officers were authorized to expend up to $100.00 for the expenses of the Membership Committee, and were authorized to pay all expenses incurred by the Association as such at the 33rd annual meeting of the Association.

Following recommendation by the Council, the following by-law was presented to the Association and was adopted by unanimous vote:

> Section G. Authorized Banks and Expenditures.
>
> Funds of the Association shall be deposited in a bank or banks approved for the purpose by the Association. Authorized expenditures from the funds of the Association shall be made by checks each of which must be signed by the President or President-elect, and the Secretary-treasurer or Assistant Secretary-treasurer.

The Lincoln Bank and Trust Co., Louisville, Kentucky, and the Evanston State Bank and Trust Co., Evanston, Illinois, were approved as depositories for the funds of the Association.

The names of 41 persons nominated for election to membership in the Association were read and by unanimous vote the Secretary was directed to cast a single ballot on behalf of the Association for the election of the persons named. The list is printed on page 773.

The selection of Atlantic City, New Jersey, as the place of meeting for the Association in 1957 was announced.

A resolution relating to federal support of dental research was presented by Dr. Hill and was adopted (p. 773). The Association voted to go on record as approving and seeking passage of House of Representatives Bill H. R. 2442, and directed that a statement be sent accordingly to the Chairman of the House of Representatives Committee on the Armed Services. All of the foregoing actions by the Association were unanimous.

The assembled members rose and stood in memory of the 5 members known to have died during the year.

The tellers reported the election of the general officers and the newly elected officers were installed. The newly elected president, Dr. Paul Boyle presented an address on Burma.

The meeting adjourned at 5:30 P.M.

At 8:00 P.M. the members of the Association and the American Association of Dental Schools met to hear an address by Dr. Harold C. Urey on "The Origin of the Solar System."

C. BOARD OF EDITORS, (MARCH 18 AND 19, 1955)

Reports on the Supporting Associates of the JOURNAL and on the handling of back copies of the JOURNAL were presented and accepted. The Supporting Associates had at that time contributed grants totaling $1,500.00. The number of Supporting Associates and the amounts contributed continue to grow. It was decided that the back issues of the JOURNAL should be moved to the School of Dentistry, Indiana University for storage.

The Board agreed that preprinting of abstracts does not constitute publication and that abstracts which were included in the preprinted abstracts should be published in the JOURNAL, subject to editorial revision. It was decided that degrees, titles, and names of sponsors should not be published and that in the future the calls for program items should state that degrees, military or scholastic ranks, and names of sponsors will not be printed.

The Board decided to request that the Association expend up to $1,000.00 from its general funds for extra pages in the JOURNAL, provided such contribution does not jeopardize the financial condition of the Association.

It was agreed that the use of consultants be left to the discretion of the Editor since all members are available for reference of articles.

D. COMBINED FINANCIAL REPORT OF THE SECRETARY-TREASURERS (EVANSTON AND LOUISVILLE) FOR THE 1954 CALENDAR YEAR

Receipts

	Evanston	Louisville	
General Fund (dues)	$ 36.00	$1,842.00	
JDR Fund (subscriptions from members)	191.00	5,864.00	
Preregistration fees for 1955 meeting		194.00	
Mosby Royalty Fund	600.00		
Interest on endowment	1.25		
Miscellaneous (collections for mimeographed copies of abstracts, 1954 meeting)	69.75		
	$898.00	$7,900.00	
Total receipts			$8,798.00

Disbursements

Evanston Office		
Clerical	$ 15.14	
Printing	182.25	
Postage	40.82	
Supplies	27.76	
Express	6.65	
Meeting expenses,* travel	246.17	
Gratuities	625.00	
Novice Award	100.00	
	$1,243.79	
Louisville Office		
C. V. Mosby Co. (block subscription to JDR)	$4,542.75	
Petty cash (cash on hand Dec. 31, 1954, $9.18)	50.00	
Clerical	15.00	
Printing, mailing operations, and postage	315.46	
Refund	7.00	
Bank tax	1.72	
	$4,931.93	
Total disbursements		$6,175.72

Bank Balances as of Dec. 31, 1954
Evanston State Bank and Trust Co.

General Fund (checking and savings accounts)	$1,260.76	
Endowment savings account	390.00	
Total	$1,650.76	
Lincoln Bank and Trust Co., Louisville		$4,332.36

Evanston books and statements audited by J. R. Blayney.
Louisville books and statements audited by Henry M. Wilbur.

Financial Report of the Publication Committee,
Business Manager, J. Frank Hall, Indianapolis

From Jan. 1, 1954, through Dec. 31, 1954.

Balance on hand Jan. 1, 1954	$313.98	
Receipts from back issues of the JOURNAL	110.50	
Total		$424.48

Disbursements

Storage and shipping charges Waverly Press, Baltimore	$75.81	
Printing	20.60	
Postage	6.16	
Miscellaneous:		
(Editor's 1953 office expenses)	$119.75	
Bank service charge	.90	
	120.65	
Total		$223.22
Balance in Bank Dec. 31, 1954		$201.26

*Including cost of 500 mimeographed copies of abstracts for 1954 meeting, $97.50.

E. BUDGETS

For the Publication Committee:

Cost of shipping back copies of JOURNAL to Indianapolis		$ 250.00
For each quarter of the year:		
Editor's office	$250.00	
Business Manager's office	125.00	
Extra pages in JOURNAL	250.00	
Total for each quarter	$625.00	
Total for four quarters		$2,500.00
Total		$2,750.00

These expenditures are not to be made from the general fund of the Association but from special funds only and are dependent upon the availability of special funds for the purpose. If more than $250.00 is available for extra pages in the fourth quarter it may be expended.

For Offices of Secretary-treasurer and Ass't Secretary-treasurer:

Direct expenses		
Clerical	$ 250.00	
Postage	150.00	
Supplies	150.00	
Printing	600.00	
Travel and Miscellaneous	50.00	
Novice Award	100.00	
Contingency fund	200.00	
Gratuity	500.00	
Total		$2,000.00

(Note: This budget does not include the cost of preprinted abstracts or their handling. The amounts in each of the categories of direct expense are approximate and transfers of funds from one category to another as necessary are approved.)

F. RESOLUTION PROPOSED BY DR. HILL AND ADOPTED BY VOTE OF THE ASSOCIATION

Whereas it is recognized that dental and oral diseases are important causes of disability, pain, and economic loss, and that the economic loss is evidenced by the fact that one of every six dollars spent for health services and care is spent for dental needs, and

Whereas the federal support to investigators of the causes and correction of dental and oral diseases is inadequate to supply the needs of workers in these fields,

Be it therefore resolved: That the North American Division of the International Association for Dental Research does hereby petition Congress to increase the funds for the support of dental research, so that increasing numbers of worthy projects can be adequately supported.

VII. OFFICERS AND MEMBERS ELECTED AND NECROLOGY

OFFICERS ELECTED: The tellers reported the election of the following general officers: President—*Paul E. Boyle,* University of Pennsylvania. President-elect—*J. F. Volker,* University of Alabama. Vice-president—*Reidar Sognnaes,* Harvard University. Secretary-treasurer—*Edward H. Hatton,* Northwestern University. Assistant Secretary-treasurer—*Dan Y. Burrill,* University of Louisville. Trustee—*Paul C. Kitchin,* Ohio State University.

NEW MEMBERS, ELECTED MARCH, 1955. *North America:* James Knuckey Avery, Howard H. Chauncey, Carl W. Fairhurst, Donald B. Giddon, Roy Gillette, Nicholas George Grand, Richard Curtice Greulich, Loren W. Gruber, Edward C. Hinds, Verda Elizabeth James, Benjamin B. Kamrin, Charles D. Kochakian, Joseph S. Landa, Dorothy F.

Mardfin, Benjamin C. Moffett, Jr., F. A. Moorrees, Lloyd J. Phillips, Irwin B. Robinson, E. Ernest Rose, Harold R. Schreiber, Adeeb E. Thomas, William Fredrick Via, Jr., Paul Vinton, Wayne Warde Wantland, Bertram L. Wolfsohn, Jack Zwemer, Eugene R. Zimmerman.

Outside North America: D. J. Anderson, England; C. H. Down, Australia; E. Desmond Farmer, England; A. D. Hitchin, Scotland; E. A. Marsland, England; Ronald Bruce Nevin, New Zealand; George Philippas, Greece; W. Stewart Ross, England; James Scott, N. Ireland; R. B. Lucas, England; Rudolf Sprinz, England; P. H. Staple, England; Albert Abraham Sulke, Israel; Robert J. Weill, France.

Total New Members Elected, 41.

NECROLOGY. *Douglas M. Beebe*, Arlington, Va. *Elmer S. Best*, Minneapolis, Minn. *J. F. Colyer*, London, England. *Harry M. Crawford*, Dallas, Tex. *Robert E. W. Hewat*, Wanganui, New Zealand.

VIII. MEMBERS VOTING AT THE ANNUAL MEETING

Afonsky, A. D. Aisenberg, M. S. Aisenberg, G. M. Anderson, Armstrong, Arnold, Bailey, Baranoff, Baume, Bavetta, Bay, Berger, Besic, Bhaskar, Bibby, Bien, Bier, Blackerby, Blackwell, Blayney, Bodecker, Boucher, Bradley, Brand, Brandhorst, Brashear, Broadbent, Brodsky, Bruckner, Brudevold, Burket, Burrill, Camalier, Campbell, Carr, Carter, Caul, Cheyne, Chilton, Clark, E. D. Coolidge, Costich, Cox, Cremer, Cross, Crowley, Curby, Dale, Darlington, Dean, Dietz, Dobbs, Dreisen, Dressel, Dummett, J. M. Dunning, Easlick, R. W. Edwards, Eggers-Lura, Ellis, Enoki, I. A. Epstein, Erikson, Everett, C. H. Fischer, T. E. Fischer, Fisher, Fitzgerald, H. S. Fleming, W. C. Fleming, Forscher, Frey, Fullmer, Gafafer, Gans, Gilda, Ginn, Glass, Glasson, Godwin, H. J. V. Goldberg, Goldsmith, Grossman, Gruebbel, Gurley, Hadjimarkos, Hahn, Haldi, Harris, Harrison, Hartles, Hatton, Hausser, Hein, J. L. Henry, Hess, Heuser, Higley, Hildebrand, I. N. Hill, T. J. Hill, Hine, Hockett, Hodge, Hoffman, Horowitz, Hunter, Hurme, Imagawa, Jasper, Jay, Jensen, Johansen, Kalnins, Kerr, Keyes, Keys, Kirino, Klaffenbach, Klapper, Klatsky, Kniesner, Knighton, Knudtzon, Koser, Koski, Kreshover, Krogh, Land, Lasater, Lazansky, Lefkowitz, Leonard, Leung, Levy, Lieban, Likens, Losee, Lovestedt, Lowery, Lowry, Ludwick, T. F. McBride, McCall, McCauley, McClure, McCrea, Mahler, Main, Mandel, Manhold, Manly, Marshall-Day, Martin, Massler, Matsumiya, Meisel, Mercado, Midgley, Millhon, Mimura, Minoguchi, D. F. Mitchell, Moen, M. Moore, N. A. Moore, A. Morse, Moyers, Mueller, Munson, H. I. Myers, R. E. Myers, Nagai, Nathans, Nelsen, Nelson, Nikiforuk, H. J. Noyes, Oartel, Ockerse, O. A. Oliver, Onisi, Ostrom, Paffenbarger, Palazzi, H. B. Palmer, Parfitt, Pearlman, Pedersen, Permar, Person, H. W. Peterson, W. A. Peterson, Pettit, Peyton, Pfeiffer, Phatak, Phillips, Pigman, Podolin, Posner, Prinz, Proel, Rabinowitch, Radusch, Rae, Ramfjord, Rault, Revess, H. B. G. Robinson, Rosebury, Rosenstein, G. D. Roth, L. H. Roth, Rovelstad, Rushton, Ruzicka, Ryge, Salley, Sartori, Sausen, Savara, Scherp, Schneyer, Schour, Scott, Selmer-Olsen, Shafer, Shapiro, Shaw, Shroff, Simon, Skillen, D. J. Smith, D. L. Smith, E. L. Smith, Smyd, Snyder, Sobel, Sognnaes, Sorrin, Spangenberg, Sprague, Sprawson, Sreebny, Stack, Stafne, Stahl, Stanford, Suhara, Suher, Swaney, W. F. Swanson, Sweeney, Sweet, Tam, Takahashi, Tebo, Teuscher, Thielemann, J. R. Thompson, H. S. Thompson, Tiblier, Trautz, Van Huysen, Volland, Von Kreudenstein, Waggener, Wallace, Walter, Ward, Warner, Wasserman, Weber, Weinmann, Wessels, Wheatcroft, White, Wilbur, Williams, Willman, Wuehrmann, Wynn, Yamada, Yamazaki Yao, Yokota, Young, Yurkstas, Zander, Zerosi, Zipkin.

IX. MEMBERS REGISTERED AND IN ATTENDANCE, LISTED BY SECTIONS

North America

Ann Arbor—Asgarzadeh, Ash, Crowley, Easlick, E. E. Henry, Jay, Mahler, Moyers, Peyton, Ramfjord. *Baltimore*—M. S. Aisenberg, Hahn, Shay. *Birmingham*—Bailey, Finn, Klapper, Kraus, Lazansky, Pigman, L. Robinson, Schneyer, Volker, Wuehrmann. *Boston*—

M. M. Cohen, Curby, Hatch, Hurme, Manly, Marshall-Day, Munson, Shaw, Shiere, Sognnaes, Turner, Turesky, Yurkstas. *Chicago*—Besic, R. E. Blackwell, R. Q. Blackwell, Blayney, Bradel, Brodie, Calandra, E. D. Coolidge, T. B. Coolidge, Dahlberg, Dean, Denton, Doty, Engel, Fosdick, Fritz, Gans, I. N. Hill, Hoffman, Jarabak, Kesel, Koser, Laskin, Massler, Medak, Molnar, Orland, Pearlman, Rapp, Schour, Schram, Skinner, Sreebny, Teuscher, Tilden, Tylman, Wach, Wallace, Weinmann, White. *Cleveland*—Bruckner, Burwasser, Chase, Dressel, Gillis, T. J. Hill, Matt, Wylie. *Columbus*—Edwards, Kitchin, Kolas, Lefkowitz, H. B. G. Robinson, Spangenberg. *Detroit*—Smyd. *Houston*—Arnim. *Indianapolis*—D. Boyd, Hall, Hine, Muhler, R. W. Phillips, Swartz, Van Huysen. *Iowa City*—Fisher, Kern. *Kansas City*—Carter, Gruebbel, H. I. Myers, G. Roth, Warner. *Louisville*—Burrill. *Minnesota*—Armstrong, Crawford, Jensen, D. F. Mitchell, Sausen, Singer, Stafne, Zander. *Nebraska*—Hooper, Waggener. *New York*—Bevelander, Brumfield, Butcher, Ellison, E. Klein, Krasnow, Levy, Neumann, Person, P. B. Taylor, Trautz. *Pacific Northwest*—Hadjimarkos, Law, A. W. Moore, H. J. Noyes. *Philadelphia*—Boyle, Burket, Cobe, Crowell, M. Moore, N. O. Taylor, Williams. *Pittsburgh*—Cox, Leung, Meisel, L. H. Roth, W. F. Swanson. *Richmond*—Clough, Knighton, Kreshover, Salley. *Rochester*—Afonsky, Bibby, Brudevold, Campbell, Costich, Hein, Hodge, Johansen, Lobene, H. M. Myers, Scherp. *San Francisco*—Agnew, S. Epstein, W. C. Fleming, Kalnins, Scrivener, Shafer. *St. Louis*—Allen, Boling, Brandhorst, Gurley, Manhold, Ring, Rosebury. *Southern California*—Bavetta. *Toronto*—Grainger, Hunter, Macdonald, Nikiforuk, Rae. *Washington, D. C.*—Arnold, G. M. Brauer, Burnett, Burstone, Caul, Dickson, Fitzgerald, Forziati, Hampp, R. Harris, Hartley, Hess, Kennedy, Keyes, Kruger, Likins, Losee, Ludwick, McBride, Paffenbarger, Posner, Rault, Rogosa, Schantz, Scott, D. L. Smith, Souder, Stanford, Stephan, Sweeney, D. F. Taylor, Zipkin.

Not Members of a Section—J. D. Boyd, J. C. Brauer, Coleman, Eichel, H. S. Fleming, Frey, Gibilisco, Goldberg, Haldi, Hunt, Manhold, Martin, T. E. Moore, Nelson, H. B. Palmer, Radike, Rovelstad, Ryge, D. J. Smith, Strader, Wynn.

Outside North America

Harvold (Norway), Rushton (England).

New Members

Avery, Chauncey, Fairhurst, Giddon, Gillette, Grand, Hitchin, James, Kochakian, Mardfin, Moorrees, L. J. Phillips, I. B. Robinson, Rose, Thomas, Via, Vinton, Wantland.

Members (including new members) registered and in attendance, 251. Guests in attendance, 361. Total attendance, 612. Members preregistered, but with no record of attendance, 20.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the British Division
London Hospital Dental School, Whitechapel, London

April 15th and 16th, 1955

Compiled by A. E. W. Miles, *Editor, British Division*

I. Index of Participants and Sequence Numericals for Corresponding Abstracts

Appleman, M.D., 19; Bradford, E. W., 13; Darling, A. I., 12; Davies, T. G. H., 24; Fearnhead, R. W., 14; Forster, M. G., 3; Freese, J. A., 19; Glasstone Hughes, S., 9; Hartles, R. L., 20; Jackson, D. M., 22; Jenkins, G. N., 3; Kerr, A. C., 21; Kramer, I. R. H., 16; Little, K., 6, 5, 4; Lucas, R. B., 17; Miller, J., 10; Milne, E. D. F., 4; Mumford, J. M., 2; Parfitt, G. J., 1; Pedersen, P. O., 24; Prophet, A. S., 23; Riera, M., 19; Rowles, S. L., 6; Scott, J. H., 23; Slack, G. L., 25; Speirs, R. L., 3; Stack, M. V., 11; Staple, P. H., 18; Symons, N. B. B., 8; Tonge, C. H., 7; Turner, E. P., 15; Wasdell, M. R., 20; Weidmann, S. M., 22.

The British Section, which now has risen to the rank of a Division, held its third meeting at the London Hospital Medical College, University of London, on Friday, April 15, and Saturday morning, April 16, 1955. Twenty-five papers and 6 demonstrations were presented and the meeting was attended by 32 members and a similar number of visitors.

1. Conditions Influencing the Incidence of Occlusal and Approximal Caries in Children. *G. J. Parfitt, Department of Preventive Dentistry, Institute of Dental Surgery, University of London.* Data was available from surveys on the incidence of dental caries in various groups of children, including 2 longitudinal surveys in which a total of 10,000 examinations were carried out, using a standard technic of examination (*Brit. D. J.* **96**: 296, 1954). Factors affecting approximal caries, such as the width of the interdental space, were studied first. Fifty-seven children, aged 4 years, showed 10 times less caries when the teeth were spaced by 0.5 mm. or more, than when the teeth were not spaced. Analysis of clinical findings and bite-wing radiographs of caries on the distal surface of the first molar (*d*) and on the mesial surface of the second molar (*e*), establishes the fact that caries is more common on the distal surface of *d*. The apparent reason for this is that a number of cavities occur on the distal surface of *d* before the eruption of *e*, and thereafter the incidence of caries on the 2 surfaces is similar. Caries on 1 surface apparently causes caries to appear on the opposing surface also. However, a greater number of cavities appear primarily on the distal surface of *d* and the cavities in *d* are in a more advanced stage of development. The distal surface of *d*, therefore, appears to be more susceptible to caries than the mesial surface of *e*, suggesting also a structural or morphologic difference between the 2 surfaces. There is evidence of independence of factors influencing occlusal and approximal caries. Caries experience in the 2 sites is not closely correlated and the yearly percentage of caries between the ages of 2 to 12 years shows a different pattern of caries experience in the 2 sites. The ratio of occlusal to approximal caries alters with age, therefore, but the ratio remains constant in individuals showing different susceptibility to caries, living under institution conditions, or in high-

or low-fluorine areas. In different national groups, where the general level of caries varies considerably due, it is suggested, to different dietary habits, the ratio of occlusal to approximal caries is markedly affected. Groups of children were examined in England, Australia, New Zealand, Singapore, and Penang.

2. Attempts to Correlate the Electrical Resistance of Teeth With the Presence and Extent of Dental Caries. *J. M. Mumford, School of Dental Surgery, University of Liverpool.* A current from a dry battery was passed through a tooth and measured by a microammeter in series. The potential drop was measured and the resistance derived by Ohm's law. The resistance from the pulp to normal areas on the outer surface of the enamel was first established by measuring in 810 cases on freshly extracted teeth. With an electrode contact of 3 sq. mm. the average resistance was approximately 8.5 megohms. The electrode was next applied to freshly extracted teeth with abnormal areas, e.g., pits, fissures, decalcification, or caries. It was found that the resistance was greatly reduced and that there was an approximate relationship between the resistance and the depth of the fissure or caries. In an attempt to establish a definite correlation, the resistance of 260 teeth was measured in vivo and related to the depth of the fissure or caries, the depth being divided into 6 levels. It was found that there was a close relationship between the depth and the average resistance of large groups but that the readings in one group overlapped those in the next. The method thus gives an approximate indication of the extent of fissures or caries and might be developed so that it will indicate whether or not caries has reached the dentin, or if the pulp has been cariously exposed. In its present form, however, it is not sufficiently accurate.

3. The Refinement of Carbohydrate and Caries. *Margaret G. Forster, G. N. Jenkins, and R. L. Speirs, King's College, Newcastle upon Tyne.* The experiments of Osborn et al. (*J. D. Res.* **16**: 165-171, 545-550, 1937; **20**: 59-69, 1941) which led to the view that unrefined sugars and flour contained a "protective factor" against caries have been repeated and extended. The results were as follows: (1) When calcium phosphate or ground enamel or dentin was incubated with saliva and either cane extracts in various stages of refinement or white or brown flour, less calcium phosphate dissolved in the presence of most of the unrefined materials. The individual constituents responsible for this effect have not been identified, but in the less refined sugar products (but not the flour) they are probably related to buffering power. (2) When similar experiments were performed with whole teeth, less tooth substance dissolved in the presence of some of the crude sugars, but no differences were found with flours of varying extraction rates. (3) Experiments with hamsters failed to show any influence of unrefined carbohydrate on caries incidence. Thus, the in vitro experiments of Osborn et al. are partly confirmed, but their biologic significance is made less likely by the negative findings in the animal feeding experiments.

4. X-ray Diffraction Methods in Dental Research. *E. D. F. Milne and K. Little, M.R.C. Staff, A.E.R.E. Harwell.* Applications mentioned were to the determination of orientation of crystallites, measurement of spacings, and identification of compounds. As an example of the second, measurements were given of strontium uptake in bone by Thewlis from which it was shown that, of an uptake of strontium of 7 per cent found in ion exchange experiments by Harrison and Raymond, only about 2 per cent was represented by a direct exchange of strontium atoms for calcium atoms in the hydroxyapatite structure. Identification of compounds by means of incineration was then discussed. Pure hydroxyapatite of composition close to the theoretical remains

as hydroxyapatite on heating to 1,000° C. When this was mixed with $CaHPO_4$ and heated, it gave a mixture of hydroxyapatite and β-$Ca_3(PO_4)_2$ in varying amounts up to pure β-$Ca_3(PO_4)_2$ depending upon the original proportions of the 2 substances present. Assuming that naturally occurring apatites behave in a similar fashion to synthetic ones, this gives an additional method for gaining information about their composition, in particular, about the amount of calcum hydrogen phosphate, presumably present as a surface layer. (Neuman and Neuman, *Chem. Rev.* **53**: 1, 1954.) Thus it has been demonstrated that growing bone contains more calcium hydrogen phosphate than established bone; that poorly formed bone, due to vitamin D deficiency, contains less calcium hydrogen phosphate than normal; that the translucent dentin which we have examined has none; and that the proportion present was unaffected by early caries in enamel. The observation by Jensen and Danø (*J. D. Res.* **33**: 741, 1954) of β-$Ca_3(PO_4)_2$ in many unheated specimens of salivary calculus was discussed.

5. Electron Microscope Studies on Teeth. *K. Little, M.R.C. Staff, A.E.R.E., Harwell.* Electron microscope pictures were shown to illustrate the following points: (1) The enamel crystallites varied in length from 2-500Å, the majority being 2-3000Å. The widths were of the order of 500Å, the thickness being somewhat less than half the width. Many crystallites from enamel treated with formic acid containing excess "calcium phosphate" were striated. This was probably an etching effect. Periodicity was in the range 350-400Å, and sometimes half this value, suggesting that crystallites are built up from smaller units of same order of size as those in dentin. The dentin crystallites were very thin plates, approximately 350Å to 400Å across, and laid down between collagen striations. (2) The softening of enamel treated with the formic acid solution was due to the removal of prism interiors. The remainder had the appearance of prism walls of variable thickness. In prisms whose contents had not been lost the arrangement of crystallites was different from that in prism walls. In the tooth shown, the angles of the 2 groups of crystallites were similar to the 5 degrees (in prism wall) and 40 degrees (in prism interior) previously demonstrated by Thewlis. (3) Owing to differences of hardness and elasticity, it is difficult to cut dentin matrix and tubules uniformly. Stereoscopic photographs showed that appearances which might be interpreted as density differences are, in fact, thickness differences. The texture of the matrix closely resembled that of articular cartilage. Typical photographs of sections of carious dentin were shown.

6. Some Observations on Histological Technics. *S. L. Rowles, M.R.C. Staff, Department of Dental Pathology, University of Birmingham, and K. Little, M.R.C. Staff, A.E.R.E., Harwell.* An investigation into the mechanism of Brain's method for the preparation of enamel for histologic sectioning has been carried out. In this method, which is based on Hardwick's physicochemical theory of caries, the tooth is exposed to a weak organic acid (formic) in the presence of excess solid "tricalcium phosphate." It has been shown that a considerable part of the mineral matter of the tooth is lost during this process. An outer layer of the enamel is much more resistant than the remainder. In parts dentin is completely demineralized. X-ray diffraction shows that the inorganic residue in enamel is predominantly hydroxyapatite. Loss of mineral occurs principally from within the prisms, and electron microscope photographs suggest that chemical solution may not be the only process involved. Part of the organic content of enamel is also lost. Solubility studies on enamel, commercial tricalcium phosphate, etc., will be reported.

7. Early Development of the Musculature of the Oral Floor. *C. H. Tonge, Department of Anatomy, Medical School, Newcastle upon Tyne.* The development of the musculature of the oral floor has been studied in coronal and sagittal serial sections of a graded series of 16 human embryos and fetuses ranging from the sixth week to the end of the fourth month. A condensation giving origin to the anterior belly of the digastric and the mylohyoid appears during the seventh week in the area between Meckel's cartilage and Reichert's bar independent of the forming genioglossus and geniohyoid muscles. As development proceeds, the anterior part of the mylohyoid is observed to lie below the level of Meckel's cartilage while its posterior and more vertical portion arises above the level of the cartilage. The intermediate area of the muscle opposite the 2nd milk molar arises from tissue on the medial aspect of Meckel's cartilage. During the fourth month the pattern is similar to that of the adult as described in earlier work (*J. D. Res.* **33**: 732, 1954). It is suggested that the space between the anterior and posterior parts of the mylohyoid corresponds to the area occupied by a section of Meckel's cartilage during development. Evidence of a fibrous raphe is seen in the part of the muscle nearer to the hyoid bone. Deficiencies in the oral floor close to the mandible seen in some specimens were filled by the anterior belly of the digastric. Vascular and lymphatic channels between the oral and submandibular surfaces were observed near the midline and adjacent to the area of closest contact between the mylohyoid and Meckel's cartilage.

8. The Cells of the Odontoblast, Ameloblast, and Internal Enamel Epithelial Layers. *N. B. B. Symons, Dental School, University of St. Andrews, Dundee.* In undecalcified formalin-fixed material from rats 2 to 4 days after birth it has been possible to confirm the presence of the kionoblast in the ameloblast layer of the developing tooth. It appears to be related by its branching distal end to the interprismatic substance of the enamel. In the same material, a cell different from but lying among the odontoblasts has been demonstrated. This cell was first described by Jasswoin (*Arch. Mikr. Anat.* **102**: 291, 1924), who called it a radial cell. The radial cell appears to be part of a system of fibers and cells, some of which lie deeper in the pulp. It is closely related to the fiber basis of the dentin matrix. This view is supported by the histochemical findings.

9. Experiments on the Calcification of Teeth. *Shirley Glasstone Hughes, Department of Anatomy, University of Cambridge.* Rosenheim and Robison (*Biochem. J.* **28**: 712-719, 1934) found that uncalcified cartilage of rachitic animals could be calcified by immersion in a solution containing organic and inorganic phosphates and calcium salts. In the present experiments whole tooth germs in various stages of development were so treated, while in others the pulp and enamel organ were removed, and the remaining caps of uncalcified dentin, or uncalcified enamel and some uncalcified dentin, were immersed in such solutions. The dentin and enamel became calcified, even in the absence of the cells in which phosphatase is present. This problem is being pursued further. Calcifiability of dentin matrix laid down in tissue culture was also demonstrated under these conditions. Calcification of whole teeth, and of sections, was demonstrated in these experiments by staining with silver nitrate, but it is hoped that more quantitative observations of degree of calcification will be obtained by using an x-ray microscope. The latter work is being done in conjunction with Dr. Nixon of the Cavendish Laboratory.

10. Neonatal Enamel Hypoplasia. *J. Miller, Department of Children's and Preventive Dentistry, Turner Dental School, Manchester.* Green pigmenta-

tion of the deciduous teeth has been known to be associated with hemolytic disease of the newborn since 1911 (Thursfield, *Proc. Roy. Soc. Med., Sect. Dis. Child.* **5**: 147, 1911). The green pigment has been shown to be biliverdin (Bevis, *J. Obst. Gynaec. Brit. Emp.* In press). Thirteen cases of hemolytic disease associated with neurological signs of kernicterus all showed marked neonatal enamel hypoplasia. A similar hypoplasia has been observed in premature infants suffering from kernicterus. The presence of a clinical physiologic jaundice in 75% of newborn children suggests that biliverdin may be associated with the "physiologic" birth line. Bessis method (Bessis, *Rev. hamat.* **2**: 114, 1947) for the production of hemolytic anemia in rats has been repeated and enamel hypoplasia has been observed. Bile pigments may be concerned in the mechanism of the enamel hypoplasia associated with some fevers.

11. Protein and Peptide Components of Human Enamel at Birth. *M. V. Stack, M.R.C. Staff, Dental School, University of Bristol.* Complete neonatal dentitions were collected and the teeth weighed to assess developmental variability. Adequate separation of enamel from the dentin fraction by the flotation method was not possible since the greater part of the dentin was more calcified than cervical enamel (specific gravity 2.0 to 2.1). Organic contents of dentin-free enamel fractions separated by flotation were correlated with the graded specific gravities of the chloroform-bromoform mixtures used. Similar amounts of enamel and dentin were present in whole dentitions of average weight within the range ½ to ¾ Gm. (15 cases). Pooled samples of the most advanced teeth, incisors and molars, were separated to give 2 parts of dentin to 3 parts of enamel, 1 part of which was of specific gravity above 2.4. The 2 enamel fractions were demineralized rapidly and dialysed. Amino acids in the fraction which diffused were determined by a modified ninhydrin-cyanide reagent. It was shown that ½ the organic matter in the lighter enamel (sp. gr. 2.2 to 2.4) was acid-insoluble protein, ⅓ was acid-soluble protein, and the remainder was diffusible peptide. The matrix of the heavier enamel (sp. gr. 2.4 to 2.6) comprised rather less insoluble protein, a similar proportion of acid-soluble protein, and somewhat more peptide fraction (*J. A. D. A.* **48**: 297, 1954).

12. The Early Lesion of Caries of the Dental Enamel. *A. I. Darling, Dental School, University of Bristol.* By means of soft x-rays of ground sections, decalcification has been shown in early enamel caries before breakdown of the enamel surface. In decalcified sections of the same lesions, it has not been possible to demonstrate any change in the organic matrix. By means of transmitted light, polarized light, and soft x-rays, a comparison has been made between ground sections of natural and artificial carious lesions and lesions produced by dilute lactic acid, each with the enamel surface still intact. All 3 types of lesions appear to be identical except for distribution which was localized in natural caries and widespread in the other 2. Each lesion consists of a central area of decalcification fading away towards the normal enamel beneath the lesion on the one hand and the enamel surface on the other. These findings are confirmed by both x-rays and quantitative measurements in polarized light. The surface enamel appears to show little or no decalcification. The margins of each lesion show, in polarized light, a "positive zone," in contrast with the usual negative birefringence of enamel. This zone corresponds to the dark zone outlining early caries lesions. It is now obvious that this zone has little significance. It is due to form birefringence and has been temporarily abolished in some imbibition media.

It now seems probable that the early attack of caries in enamel is predominantly a decalcification by agents which probably gain access through a small entry in a more or less resistant enamel surface, spreading fairly easily in all directions in the underlying enamel. Later it probably strikes back through the enamel surface. Work is still proceeding on the nature of the entry, the precise significance of the positive zone, and the role of normal structure and developmental faults in the spread of the lesion.

13. Some Observations on the Attachments of Teeth. *E. W. Bradford, Dental School, University of St. Andrews, Dundee.* By reference to the following species. (*Scyliorhinus caniculus, Raia batis, Gadus aeglifinus,* and *Labrus bergylta*) there is a basic pattern of distribution of tissues in the tooth. Each tooth is formed by an outer layer of highly calcified tissue and an underlying body of dentin. This latter is differentiated from the tissue of attachment or so-called bone of attachment by an uncalcified hinge. This is true in the chondrichthyes as well as in the osteichthyes. This whole denticle is then attached to the jaws either by a fibrous ligament in the chondrichthyes or by ankylosis in the osteichthyes. In view of the similarity in the construction of the denticles in these 2 large sub-classes of pisces, and the fact that the variation in attachment of the tooth actually occurs between the tissue of attachment and the jaw, it is suggested that the issue of attachment should really be considered to be a dental tissue rather than one of skeletal origin.

14. Observations on the Innervation of Dentin. *R. W. Fearnhead, Department of Dental Histology, The London Hospital Medical College.* Using a modified version of Holmes' silver technic, control over the demonstration of nervous elements in dental tissues has been obtained. The optimal staining depends upon the degree of fixation and the fixatives used. The results on decalcified sections are constant and give a clear well-differentiated impregnation. Ramification of nerves in the pulp and the passage of their branches between the odontoblasts, loops of fine nerves in the predentin, and the presence of fine beaded fibrils forming a marginal plexus have been confirmed. Numerous very fine branches from the marginal plexus pass a considerable distance into the dentinal tubules well past the predentin. An attempt was made to test the accuracy of these observations in the light of recent advances in knowledge of the structure of dentin.

15. Calcified Repair Tissue of Teeth. *E. P. Turner, Dental Department, University of Manchester.* In many pathologic conditions where tooth substance is lost there is an attempt at repair by the adjacent connective tissue; this may result in the formation of calcified tissue which does not resemble the resorbed tissue. An example may be seen in internal resorption of teeth, where tissue resembling bone replaces the resorbed dentin and enamel. Osseous tissue has been found replacing resorbed areas in the crowns of some specimens of unerupted teeth. The reduced enamel epithelium of these specimens was scanty and it may be that in the areas where it broke down, enamel resorption commenced. Tissue resembling primitive bone has been found lining the pulp canals of teeth in which there is a pulp polypus. In this instance the mesenchymal cells of the pulp may have been stimulated to form the tissue by a low-grade toxic infiltration. Similar tissue has been found on the dentin surface of un-united fractures of the roots of permanent incisors. In young children where there has been early loss of the deciduous incisor crowns due to rapid caries a barrier of tissue sometimes forms across the exposed pulp chamber, especially if root development is incomplete and the blood supply to the pulp

is abundant. In some cases this barrier sometimes resembles the "osteodentin" described by Fleming (*Oral Surg., Oral Med. & Oral Path.* **6**: 1315-1324, 1953) which occurs as a metaplasia of the dentin of transplanted tooth germs.

16. The Development of Characteristic Changes in the Dental Pulp Following the Use of Resins Containing Methacrylic Acid. *Ivor R. H. Kramer, Department of Dental Pathology, Institute of Dental Surgery, University of London.* Using methods previously described (Kramer and McLean, *Brit. D. J.* **92**: 255-261, 281-287, 311-315, 1952; McLean and Kramer, *Brit. D. J.* **93**: 255-269, 1952; Kramer, *Brit. D. J.* **96**: 9-13, 1954) the effects on the human pulp of 2 further self-polymerizing acrylic resins were investigated. In addition to inflammatory cell infiltration, aspiration of cells into dentinal tubules, and secondary dentin formation, large edema "blisters" were found on a number of occasions. This type of reaction, not encountered with any of the other materials tested, was characterized by the formation beneath the odontoblast layer of multiple small blisters, single large loculated blisters, or large unloculated lesions. The inner surface of the dentin in the area affected was clothed with a layer of compressed pulp tissue. It was found that these lesions could develop within 1 day of filling and might be found as long as 42 days after filling. Methacrylic acid is the only constituent of these resins not shared by other materials which do not cause edema blister formation, and it is concluded that this form of pulp reaction is attributable to the methacrylic acid. The incidence was higher with Material A than with Material B, and evidence was found that the higher incidence was due to variations in the methacrylic acid content of the mix. However, reduction to half the methacrylic acid concentration so prolonged the setting as to make the material unsuitable for clinical use. The absence of any obvious reduction in blister incidence following reduction in methacrylic acid concentration may be attributable to persistence of the methacrylic acid for longer in the slower setting mixture.

17. The Effect of Aureomycin on the Pyridoxine-deficiency Periodontitis of Mice. *R. B. Lucas, Royal Dental Hospital of London.* Levy (*J. D. Res.* **29**: 349, 1950) reported that mice maintained on pyridoxine-free diets developed inflammatory lesions of the periodontal tissues, particularly if the diet, at the same time, had a high protein content. In an attempt to reproduce such lesions, and to determine the effect of Aureomycin on their development, 60 mice (28 days old, M.R.C. stock) were divided into 4 groups, A, B, C, and D, and placed on the synthetic diets described by Levy. Groups A and B received low protein (18 per cent) diets, with the addition of Aureomycin (30 ppm) in the case of Group B, while Groups C and D received high protein diets (54 per cent) deficient in pyridoxine. Group D received Aureomycin in addition (30 ppm). All diets were given ad libitum. Animals from each group were killed at 2 weekly intervals, the whole experiment being run for 10 weeks. The mandibles and maxillae were decalcified and serial sections prepared. The changes described by Levy included acute and chronic inflammatory infiltration of the epithelium of the interdental papillae, with its ultimate ulceration and destruction after 8 weeks on a pyridoxine-free diet. There was also recession of the alveolar bone, and the epithelial attachment migrated apically. These changes were more severe when the protein content of the diet was high.

In the present experiments, changes in the interdental papillae comparable with those described by Levy at the 2-week stage were noted in those animals of Groups C and D killed at 4 weeks, and similar lesions, although not more

extensive, were present in the remaining animals of those groups, killed at later stages. The lesions took the form of scanty inflammatory infiltration and necrosis of the superficial layers of epithelium, with no involvement of the deeper layers or of the subepithelial tissues, and were found in approximately 75% of animals of the pyridoxine-free groups. Since these lesions occurred in the animals of both Groups C and D, it is concluded that the addition of 30 ppm of Aureomycin to the diet does not prevent their development.

18. Observations on the Gingival Capillary Circulation in Human Subjects. *P. H. Staple (External Staff, Medical Research Council), Inter-Departmental Laboratory, Guy's Hospital, London.* Serial observations on the same area of tissue have been made for up to 4 months in 3 men (aged 21, 34, and 39 years) and 2 women (aged 21 and 25 years) using a modified version of the apparatus described by J. D. King (*D. Rec.* **67**: 3, 1947). Most observations concern the labial marginal gingiva of $\overline{3|3}$, but $\underline{3|}$ and $\overline{2|}$, and the interdental papillae of $\overline{321|}$ have also been studied. The tissues were clinically normal, and the subjects were healthy, consumed a good mixed diet, and carried out efficient oral hygiene. Under these circumstances (control period) the capillary pattern of the free and immediately adjacent, attached, gingiva remains remarkably constant. Changes in the amount of blood passing through the capillary bed appear to be achieved by varying the flow through a given number of channels and not by varying the number open at any instant. The capillary pattern may differ on either side of the mouth and is an individual characteristic. After control periods, a chronic marginal gingivitis has been induced, during which the following occurs: (1) Momentary interruptions in flow of red cells in a few capillary loops. (2) These interruptions last longer, more capillaries become involved, and eventually the phenomenon resembles blood sludging (Knisley et al., *Science* **106**: 431, 1947). (3) Pari passu increased blood flow with capillary dilatation is followed by marked slowing of capillary blood flow due to blood sludging. During recovery, most of these changes are reversible. Except once when permanent stasis occurred, no additional capillaries have been seen functioning during experimental gingivitis.

19. A New Technic for Studying the Flora of the Tooth. *M. D. Appleman, J. A. Freese, and M. Riera, Department of Bacteriology and School of Dentistry, University of Southern California, Los Angeles, Calif.* Efforts in the past to study the sequential flora of the tooth have been unsuccessful. Blayney, et al. (*J. D. Res.* **15**: 326, 1935) observed plaque structures while examining thin ground sections of human teeth. Ennever et al. (*J. D. Res.* **27**: 597, 1948) inserted dentures with attached ponties in the mouth but the sequential development of the flora of the tooth was not studied in this way. Examinations of saliva, mouth washings, or scrapings from the teeth were also unsuccessful since the first 2 do not indicate the flora of the tooth and the last shows nothing as to plaque formation. We have devised a new technic for the study of the dental flora based on a method originally used by Black (*Special Dental Pathology*, ed. 3., Chicago, 1924, *Medico-Dental Publicity Co.*), for the study of salivary calculus deposits. Small stainless steel brackets were welded to stainless steel bands which were fitted and contoured to the tooth, removed and arc-welded at the slight overlap, and finally cemented in place. Small vinylite plastic slides were inserted in the brackets. At appropriate intervals the slides were removed, gram-stained, and examined. Experiments have shown that the dental plaque is predominantly composed of Micrococcus and Streptococcus. It is quite possible that caries may be

caused by acid-producing members of the latter genus. The fact that the genus Lactobacillus was encountered only in relatively few instances supports the belief that these organisms may be of minor importance. The plaque did not start with the growth of filamentous fungi and Actinomyces on food particles, but in areas where squamous epithelial cells, and possibly other materials, are deposited and not removed. These materials are first attacked by species of Micrococcus and Streptococcus. Other elements of plaque formation occur later.

20. A Comparison of the Metabolic Activity of Salivary Sediment With That of Whole Saliva. *R. L. Hartles and Marie R. Wasdell, Biochemistry Department, School of Dental Surgery, University of Liverpool.* Sreebny, Kirch, and Kesel (*J. D. Res.* **29**: 506, 1950) reported that salivary glycolysis was due to the salivary sediment. It was demonstrated by Hartles and Wasdell (*Brit. D. J.* **97**: 231, 1954) that the supernatant from centrifuged saliva neither respired nor contained a glycolytic system. We have investigated the relationship between the metabolic activity of sediment and its containing fluid. It has been found that, while the intrinsic activity of salva rests in the solids, the fluid contains an adjuvant, in the absence of which the metabolic processes are greatly retarded. Washed suspensions of salivary solids have a much reduced activity. The addition of salivary supernatant fluid to the suspension restores the activity.

21. Continuous Recording of the Rate of Flow of Parotid Secretion in Man. *A. C. Kerr, Department of Dental Medicine, Guy's Hospital, University of London.* Various suction cups have been described for collecting the secretion of the human parotid gland. Cannulae are not generally used, on the grounds that they are dangerous, difficult to retain in position, and prone to leakage. It has been found possible to replace the central chamber of the Lashley cup (*J. Exper. Psychol.* **1**: 461-493, 1916) by a fine polyethylene cannula, thus overcoming these difficulties. This flanged cannula has been worn by 15 persons without undue discomfort or interference with oral function for periods of up to 4 hours. Lipiodol has been injected under pressure through the cannula into the duct but x-ray examination failed to reveal any leakage of Lipiodol between the cannula and the duct opening. The secretion was conducted into a device which inscribes on a drum, for each minute, a vertical line, the length of which is proportional to the total volume of secretion collected during that minute. The latter has the following advantages over conventional drop counters: (a) it gives an immediate measure of volume; (b) it is not affected by streaming of drops; (c) the records are concise and easily read; (d) it will record flow rates within the range 0.05 to 3.0 ml. per minute without adjustment. With this technic the responses of human parotid glands to various types and strengths of stimulation have been recorded. Samples of pure parotid secretion have been obtained for an investigation of the protein content and for other analyses. The relation between mastication and parotid function is being studied in detail. Two general conclusions are evident. These are (1) measurement of salivary gland flow rates that do not take into consideration that the concomitant afferent stimulation are of little value; (2) studies of the composition of salivary secretions must be related to the state of functional activity of the glands producing the secretions..

22. The Effect of Fluorine on the Mineral Metabolism of Phosphorus in Bones and Teeth of Rats. *D. Jackson and S. M. Weidmann, Biological Research Unit, School of Dentistry, University of Leeds.* Various concentrations

of fluorine in the form of sodium fluoride solution were administered in drinking water to adult rats for different periods of time. The animals were injected with labeled phosphorus and sacrificed after 4 hours by exsanguination. The cortical and cancellous fractions of the femur and tibia were compared with the growing incisor teeth in regard to fluorine uptake. It was found that phosphorus uptake was gradually reduced with increasing bone fluorine content. Under the experimental condition a maximum reduction of 50 per cent was observed without any macroscopic or radiographic evidence of skeletal fluorosis, and without any macroscopic evidence of damage to the kidney, liver, or thyroid.

23. Histologic Investigation of Cleft Palate in Tiger, Dog, and Man. *J. H. Scott, Department of Anatomy, University of Belfast, and A. S. Prophet, Turner Dental School, University of Manchester.* Serial sections were cut and stained from the heads of a newborn tiger with a palatal cleft extending to the incisive foramen region, a newborn boxer pup with a palatal cleft and a double harelip (with a normal littermate for a control), and of a full-term human fetus with a palatal cleft and a unilateral harelip. The altered relationships of structures such as the premaxillary and vomer bones, the cartilage of the nasal capsule, the nasopalatine ducts and Jacobson's organs, and the developing tooth germs will be described. An account was given of the site of union between nasal and oral epithelium.

PAPERS READ BY TITLE

24. Replica Studies of Enamel Surfaces of Deciduous Teeth. *T. G. H. Davies and P. O. Pedersen, Royal Dental College, Copenhagen.* A total of 1,812 replicas was taken from the enamel surfaces of 604 deciduous teeth and shadowed in vacuo with silver at an angle of 30 degrees. The majority were from modern Danish children and ancient Danish skulls. The smaller groups were from modern Greenland children and ancient Greenland skulls. In addition to all the structures previously described on the enamel surfaces of the permanent teeth, new structures, "microelevations," were noted. A quantitative comparison of the incidence of the structures recorded revealed no significant differences between the 4 groups.

25. The Effect of Topical Application of Stannous Fluoride Solution in the Prevention of Dental Caries. *Geoffrey L. Slack, School of Dental Surgery, University of Liverpool.* The first permanent molars of the right side were treated after prophylaxis with a 2% solution of stannous fluoride on 3 occasions during a period of 3 weeks. A fresh solution was prepared for each session (half-day). The left side acted as control. After 1 year the 328 children still available from the initial attendance of 423 were re-examined. The first permanent molars on the left side had 190 cavities which increased to 443 after one year, as compared with the treated right side which showed an increase of 190 to 351 cavities. Topical applications were repeated at the end of the first year. Examinations will be carried out again at the end of the 2-year period.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the New York Section
New York Academy of Sciences, April 27, 1955

Compiled by Frances Krasnow, *Editor, New York Section*

1. Drinking Habits of the Younger Child. *H. H. Neumann, School of Dental and Oral Surgery, Columbia University, New York, N. Y.* Histories were taken regarding the fluid intake of 312 children below the age of 7, in suburban communities on Long Island. The histories were obtained from their mothers who were unhesitant about the drinking habits of their smaller children and about the amounts used daily. Previously published figures were either based on assumptions, theoretical calculations, and on statistics of adolescents and adults. The results of the present inquiry showed a very definite pattern which can be summed up as follows: The bulk of the daily liquid requirement is provided almost entirely by milk and water, the average intake of which was 1,136 c.c. For the child from birth to age 6, the principal source of fluid intake is milk, with an average of 840 c.c. per day. The average water intake was 294 c.c. per day. In contrast to the milk intake, the water consumption varied widely, at a ratio of 1:7.4, relating the lower and the upper third. Outside of milk and water the only other fluid consumed habitually and regularly was orange juice. Sixty-six per cent of the children drank prepared frozen orange juice, 26% fresh orange juice. Other juices, as apple, tomato, and grapefruit juice, were rarely used with great regularity. During 2 months of the year, that is during hot weather, the fluid intake is affected, whereby the milk drinkers seem to increase their milk consumption while the water drinkers increase their water intake.

2. Histopathology of Some Lesions of the Tongue. *George Stein, New York, N. Y.* Histopathology of the geographic tongue, the coated tongue, and other tongue conditions in nutritional deficiencies will be discussed. Sequence of tongue changes developing from nutritional deficiencies in man and their disappearance after introducing a normal diet will be demonstrated by Kodachrome slides.

3. Topical Application of Antibiotics and Enzymes. *Alexander Seelig, Dental Department, Montefiore Hospital, New York, N. Y.* To determine the efficacy of the application of antibiotics and enzymes upon experimentally produced infections in the pulp, a series of experiments were carried out on the teeth of the rhesus monkey. It was determined that superficial infections could be controlled by the direct application of antibiotics. Deep infections were not irradicated by the topical application of antibiotics. When irrigating fluids and enzymes were used to wash away the necrotic tissue caused by the infection, the antibiotics came in contact with the pathogenic organisms and the infection was irradicated in 50% of the cases.

4. Experiments on the Regeneration of Pulp in Monkey's Teeth. *Robert V. Vidair and Earl O. Butcher, Department of Anatomy, New York University College of Dentistry, New York, N. Y.*

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the Pacific Northwest Section

University of Washington, School of Dentistry, Seattle, Washington, May 2, 1954

Compiled by A. L. Ogilvie, *Secretary-Treasurer, Pacific Northwest Section*

1. A Study of Tissue Reaction to Titanium Implants (A Preliminary Report). *Oscar E. Beder, University of Washington, School of Dentistry, Seattle, Wash.* The object of this investigation was to determine the possibilities of titanium as an implant material in surgical procedures similar to those employing tantalum. The rationale for the selection of titanium had been its properties of lightness, strength, workability, and corrosion resistance. Four autoclaved metal (1 cm. diameter) disks, (tantalum, silver, phosphobronze, and titanium), were implanted aseptically at 4 different sites between the external and internal oblique muscles of a gray male shepherd dog. The weight of the animal at the time of surgery was 13.5 kilograms. The disks were sutured with No. 30 black silk. Three of the wounds healed rapidly. A small draining sinus persisted from the site of implantation of the titanium. The disks and the surrounding tissues were excised 134 days postimplantation. The results were inconclusive because there was infection in 3 of the implantation sites. The plan is to repeat the procedure with small disks of tantalum and titanium in a greater series of smaller animals.

2. The Direction of Enamel Rods of Primary Molars as Applied to Cavity Preparation (A Preliminary Report). *Stanley L. Shephard, University of Washington, School of Dentistry, Seattle, Wash.* A study is under way to demonstrate the direction of the enamel rods in primary molars as these rods relate to cavity outline. The enamel rod direction is being determined by the use of photomicrographs of ground sections of primary molars. The angle of direction of the enamel rods of primary molars varies only slightly from specimen to specimen. This variation in the direction of enamel rods is considered by the author to be insignificant in its application to cavity preparation. There are no enamel rods in primary molars which are apical in their direction. Semischematic drawings and the actual photomicrographs will be made for use in the demonstration of the enamel rods as they apply to cavity preparation.

3. The Incidence of Gingivitis in Adolescents at Marysville, Washington. *Doris J. Stiefel, University of Washington, School of Dentistry, Seattle, Wash.* A survey was conducted of 402 junior high and high school students at Marysville, Washington, to determine the incidence of gingivitis and its relation to other factors. The PMA index was used for the evaluation of gingivitis. While 75.9% of the entire group was found to have 1 or more areas of gingival inflammation, the majority of affected individuals exhibited the milder categories of gingivitis only. Papillary gingivitis was found more frequently than marginal, which, in turn, occurred more frequently than involvement of the attached gingiva. The maximum incidence of gingivitis was noted at 13 to 14 years of age. Fewer girls than boys were affected with gingivitis and their severity of involvement was less. The girls also tended to show better hygiene, to brush

their teeth more frequently, to improve in brushing habits with age, and to have less gingival enlargement than the boys. The degree of oral hygiene was found to be positively correlated with the severity of gingivitis. The frequency of toothbrushing, caries, and gingival enlargement showed a possible slight correlation with gingivitis, while calculus, open bite, and the method of toothbrushing appeared to have no definite relationship to the gingival condition in this group. A comparison with studies in the eastern part of the United States indicated a high incidence of gingivitis, but a less severe degree of involvement for the Marysville group.

4. Preliminary Report on a Project to Evaluate the Keratotic Lesions of the Mucous Membranes of the Oral Cavity. *Francis V. Howell, University of Oregon Dental School, Portland, Ore.* Hyperkeratosis and dyskeratotic lesions are diagnosed clinically with much confusion and considerable damage to the patient. Histologically, changes which differentiate dyskeratosis from hyperkeratosis of the upper epithelium are to be evaluated. The freezing-drying technic similar to that developed by Gersch is to be used in this project. Biopsies from keratotic lesions in the mouth and several lesions from the female cervix will be prepared by the common formalin fixation method as well as the freezing-drying technic. Particular emphasis is being placed upon the keratohyalin granule which is present in the skin, but is absent in the mucous membrane. The keratohyalin granule is seen both in hyperplasia and dyskeratosis of the mucous membrane. The effects of salicylic acids are to be investigated. These compounds will be applied to the mucous membranes before biopsy.

5. New Silicone Compositions in Dental Practice (A Preliminary Report). *Nilkanth Phatak and Bhim Sen Savara, University of Oregon Dental School, and Jack Dement, Portland, Ore.* Present-day chemical-type dentifrices have a transient action which in itself is a self-defeating situation. The principles of physics provide sound approaches to effective dentifrice action at the enamel-saliva interface. These principles are fulfilled by use of silicone in dental therapeutics. Silicones are organopolysiloxane polymers which are chemically inert, water-repellent, insoluble in most solvents, tasteless, and of very low biologic toxicity. Silicones can wet almost any surface but are not wet by water. This new principle involves a true solution of silicone in a suitable nontoxic coupling solvent that is concurrently soluble in saliva. This silicone solution results in the lay-down of a microthin silicone film upon an essentially moisture-free enamel surface, caused by the solvation of the coupling solvent and the saliva at the interface. We have used such silicone solutions for preparing dentifrices and also chewing gums (including bubble gum) which are water-free and contain no detergent or sugar. Severe accelerated etch tests, using 10% HCl microdrops, were made upon adjacent control and treated enamel areas of the same tooth, with the appearance of gas bubbles as the end point. In a series of 64 tests (the same tooth surface serving as its own control), the mean etching time for the control surface areas was 20.8 seconds (range 9.0 to 46.2 seconds) whereas the mean for the test group was 9 minutes, 48 seconds (range 1 minute, 38 seconds to more than 24 minutes at which time observation was discontinued.) The mean difference in etching time between the control and the test tooth surfaces was 9 minutes, 45 seconds (standard deviation 3 minutes, 5 seconds, standard error 59 seconds.) This ratio should be much greater for weak acids (e.g., lactic acid), which do not directly attack the siloxane bond. In addition, 30 simulated saliva turnover tests made by allowing water to run continuously over silicone-treated teeth

showed the silicone film still remaining after 45 days. Silicone films, being hydrophobic, may reduce leakage of restoration margins, and may also exert a release action against the adherence of food debris, plaques, and staining matter to the enamel surface.

6. The Effects of Irradiation of the Pituitary Gland on the Growth of Kittens (A Preliminary Report). *Bhim Sen Savara, University of Oregon Dental School, Portland, Ore.* Earlier work in this field has been done under the direction of Herbert Evans and associates who have studied the growth changes in hypophysectomized rats. A report has also been published by Kerr in 1948, reporting on the irradiation of pituitary tumors in 50 cases. Four kittens, littermates, were used for this investigation. Two were kept as controls, while 2 others were given irradiation to the pituitary in doses of 1,000r every 3 days until one had received 3,000r and the other had received 5,000r. Roentgenograms of the whole body of each kitten were taken at the beginning of the experiment and at 2-month intervals for 5 months, at which time the animals were sacrificed. Both the experimental kittens stopped gaining weight after they had received 2,000r each. Both lost hair at the temples where the roentgen rays were directed. The hair grew back in about 2 months. The kittens did not eat for about 2 weeks after irradiation and lost considerable weight. After 2 weeks they started eating slowly and gained weight. The experimental kittens still were underweight as compared with the controls 5 months later. The roentgenograms of the teeth did not show any defects in calcification of the teeth. Eruption of the teeth was not affected. The growth of the long bones was affected slightly in comparison with the controls. However, the variation was so slight that it was considered insignificant and was most probably produced due to starvation during the first 2 weeks when the experiment was started. No scars indicating any arrest in growth of the long bones were observed. The animals were otherwise normal and did not exhibit any symptoms which may have indicated damage to the brain. The cause of lack of arrest of growth in the kittens can only be explained on the basis that the effects of irradiation to the pituitary gland were transitory. It appears that the amount of irradiation required to arrest growth in a normal tissue is much greater than that used for pathologic tissue and would most probably kill the healthy animal before an arrest in growth would become apparent.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Chicago Section

SCIENTIFIC PROGRAMS FOR 1953-1954

Compiled by G. B. Denton, *Editor, Chicago Section*

At the five meetings of the Chicago Section held during the year 1953 to 1954, the following 15 papers were presented:

October 30, 1953—Giant Cell Tumors. *Patrick Toto, Chicago College of Dental Surgery, Loyola University. Discussion opened by J. P. Weinmann.*

Inhibition of Acid Action by Various Fluoride Salts Applied Topically to Intact Human Dental Enamel. *Harbans Bhatia, University of Illinois College of Dentistry and Chicago College of Dental Surgery, Loyola University. Discussion opened by B. Gurney.*

Saliva Studies of Calculus-Free and Calculus-Positive Individuals. A. Clinical Studies, *Charles Belting, Veterans Administration Outpatient Clinic;* B. Chemical Studies, *B. F. Gurney, Chicago College of Dental Surgery, Loyola University. Discussion opened by G. Rapp.*

November 20, 1953—The Characteristics of a Proteolytic Enzyme From the Submaxillary Salivary Glands. *Leo Sreebny, University of Illinois College of Dentistry. Discussion opened by F. Orland.*

The pH and Buffering Capacity of Dental Plaques. *L. S. Fosdick, Northwestern University Dental School, and W. J. Carter, Great Lakes Naval Training Station. Discussion opened by B. Gurney.*

The Reaction of the Gingiva to Abrasive Injury. *J. A. Kollar and F. M. Wentz, Chicago College of Dental Surgery, Loyola University. Discussion opened by H. Sicher.*

January 29, 1954—The Evanston Dental Caries Study. XII. A Survey of Lactobacillus Counts With Reference to Untreated Carious Surfaces Before and After Exposure to Fluoridated Water. *I. N. Hill, Zoller Memorial Clinic, University of Chicago. Discussion opened by F. Orland.*

A Concept of Bone Formation and Resorption. *H. Sicher, Chicago College of Dental Surgery, Loyola University, and J. P. Weinmann, University of Illinois College of Dentistry.*

April 30, 1954—Distribution of Species of Lactobacilli in Extracted Teeth. *Helen Kristufek, Northwestern University Dental School. Discussion opened by E. Tilden.*

Electrometric Studies of Human Gingiva in Pregnancy. *B. J. Gans, Chicago College of Dental Surgery, Loyola University, and M. B. Engel, University of Illinois College of Dentistry.*

EXPERIMENTAL STUDIES ON THE EFFECT OF FILLING MATERIALS ON THE PULP OF THE RAT INCISOR. *M. Massler, M. Silberkweit, I. Schour, and J. P. Weinmann, University of Illinois College of Dentistry.*

PATTERNS OF CORRELATION IN TOOTH DEVELOPMENT IN THE FROG. *R. Gillette. Discussion opened by J. P. Weinmann.*

May 21, 1954—THE PATHOGENESIS OF RADIO-OSTEONECROSIS IN THE MONKEY. *Joseph M. Gowgiel, Zoller Memorial Clinic, University of Chicago. Discussion opened by J. R. Blayney.*

THE ERUPTIVE MOVEMENT OF THE FIRST MOLAR OF THE RAT. *Cyril O'Brien, University of Illinois College of Dentistry. Discussion opened by S. N. Bhaskar.*

SOME IMPLICATIONS OF ELECTROMYOGRAPHIC RESEARCH. *Harold Perry, Northwestern University Dental School. Discussion opened by S. C. Harris.*

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the Chicago Section, 1954-1955

Compiled by G. B. Denton

At the five meetings of the Chicago Section held during the year 1954 to 1955, the following fourteen papers were presented:

October 29, 1954.—(1) Tissue Changes in Traumatic Occlusion. *Balint Orban, Loyola University College of Dentistry, and S. N. Bhaskar, University of Illinois College of Dentistry.* (2) The Experimental Production of Sarcomas by Methyl Methacrylate Implants. *Daniel M. Laskin, I. B. Robinson, and J. P. Weinmann, Department of Oral and Maxillofacial Surgery, University of Illinois College of Dentistry.* (3) Effect of Sucrose on the Solubility of Human Dental Enamel. *I. Q. Blackwell and L. S. Fosdick, Department of Chemistry, Northwestern University Dental School.*

November 19, 1954.—(1) Pseudomonas Aeruginosa, a Constant Resident of the Oral Cavity in a Group of Students. *Evelyn B. Tilden, Robert C. Worley, and Patricia Gavin, Department of Bacteriology, Northwestern University Dental School.* (2) The Current Status of the Epithelial Attachment. *Harry Sicher, Department of Anatomy, Loyola University College of Dentistry.*

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April 29, 1955.—(1) The Influence of Nasal Structures on Cleft Palate Speech. *T. M. Graber, Department of Orthodontics, Northwestern University Dental School.* (2) Salivary Proteolytic Enzymes. *Thomas B. Coolidge, Zoller Dental Clinic, and Lois Lightfoot, Department of Biochemistry, University of Chicago.* (3) Alterations in Dental Tissues Following Beryllium Carbonate Feeding. *Frank Wentz and Harry Sicher, Loyola University College of Dentistry, and J. P. Weinmann and Isaac Schour, University of Illinois College of Dentistry.*

May 20, 1955.—(1) THE EFFECT OF SALIVA ON THE pH AND LACTATE CONCENTRATION OF DENTAL PLAQUES. *H. R. Englander and K. C. Hoerman, U. S. Naval Training Center, Great Lakes, and L. S. Fosdick, Department of Chemistry, Northwestern University Dental School.* (2) MUSCULAR SPASMS COINCIDENT WITH OCCLUSAL AND TEMPOROMANDIBULAR JOINT DISTURBANCES. *Joseph R. Jarabak, Loyola University Dental School.* (3) A RADIOGRAPHIC EVALUATION OF THE TEMPOROMANDIBULAR JOINT IN EDENTULOUS SUBJECTS. *Sheldon Rosenstein, Department of Orthodontics, Northwestern University Dental School.*

Addendum

In the article by Richard C. Greulich and C. P. Leblond, entitled "Radioautographic Visualization of the Formation and Fate of the Organic Matrix of Dentin," which appeared in the December, 1954, issue of the JOURNAL, pages 859-872, the following addition should be made: On page 869, in the fourth paragraph of text material, in line 11, the sentence ending ". . . to consist mainly of collagenous material.[14]," should be followed by a footnote reading, "However, the presence of mucopolysaccharides in predentin has been suggested by Verne and his co-workers,[15, 16] on the basis of evidence contrary to that presented here. We have been unable to explain this discrepancy."

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